Synthesis, Characterization, Biological and Catalytic Studies of Tridentate Monothiosemicarbazone Palladium(II) Complexes

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Master of Science

by

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Declaration

I know the meaning of Plagiarism and declare that all of the work in the document, **"Synthesis, Characterization, Biological and Catalytic Studies of Tridentate Palladium(II) Thiosemicarbazone Complexes"**, save for that which is properly acknowledged by means of references, is my own.

_____ Date:_____

Ms Prinessa Chellan

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Abstract

Nine monothiosemicarbazone ligands were prepared by Schiff-base condensation reactions of thiosemicarbazide and the appropriate aryl aldehyde or ketone. These compounds were isolated as air- and moisture- stable solids and were characterized using NMR and IR spectroscopies, as well as mass spectrometry and elemental analysis in the case of ligand 3-*tert*.-butyl-2-hydroxy-benzaldehyde thiosemicarbazone, which is a new compound.

Four of these ligands were reacted with precursors of type $Pd(L)_2Cl_2$ yielding eight tridentate thiosemicarbazone Pd(II) complexes with general formula [Pd(^{3}-O,N,S-thiosemicarbazone)(L)] (L = PPh_3 or 4-picoline). Six of these complexes are new compounds and were fully characterized using NMR and IR spectroscopies, mass spectrometry and elemental analysis. In addition, the solid state structures of three complexes were elucidated using single crystal X-ray diffraction methods.

Reaction of the thiosemicarbazone ligands with $K_2[PdCl_4]$ yielded either tetranuclear tridentate [C,N,S] complexes, formed by cyclometallation of the *ortho*-carbon, imine nitrogen and thiolato sulfur with metal centers linked via Pd-$S_{bridging}$ bonds to give a tetrameric structure or dimeric complexes, where the ligand coordinates to palladium as a singly negative bidentate [N,S] donor and each metal center is bridged by two chlorine atoms. The bridging bonds in these precursors were cleaved with the tertiary phosphine PPh_3 to yield monomeric cyclometallated complexes. All of these complexes are new compounds and were fully characterized using standard spectroscopic, spectrometric and analytical techniques. The molecular structures of one tridentate and one bidentate thiosemicarbazone Pd(II) complex were solved using single crystal X-ray diffraction and confirm the structural nature of the mononuclear complexes synthesized.

Selected mononuclear complexes and their corresponding free ligands were screened for preliminary biological activity as anticancer and antimalarial agents. Two complexes exhibited good cytotoxic activity against most cancer cell lines screened, while a free ligand was found to be the most potent inhibitor out of all compounds tested. Against the *P. falciparum* strains, none of the free ligands were active at the highest concentration tested while two complexes showed superior antiplasmodial activity against the chloroquine sensitive (D10) strain.

Catalytic studies using selected palladium(II) complexes show that tridentate thiosemicarbazone Pd(II) complexes successfully catalyze Suzuki-Miyaura carbon-carbon coupling between phenyl boronic acid and two aryl bromide substrates in the presence of water and in air. Moderate to good percentage conversions were obtained with the complexes exhibiting selectivity towards the 4-bromo-anisole substrate.

Publications

Journal Article:

Tameryn Stringer, Prinessa Chellan, Bruno Therrien, Nelusha Shunmoogam-Gounden, Denver T. Hendricks and Gregory S. Smith, *Synthesis and structural characterization of binuclear palladium(II) complexes of salicylaldimine dithiosemicarbazones,* **2009**, doi:10.1016/j.poly.2009.06.026.

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- Oral Presentation: Prinessa Chellan, Gregory Smith and Kelly Chibale, *Synthesis and Characterization of Salicylaldimine Thiosemicarbazone Palladium Complexes*, presented at the Cape Organometallic Symposium (COS), Cape Town, South Africa, 2008.

- Poster Presentation: Prinessa Chellan, Gregory Smith and Kelly Chibale, *Synthesis, Characterization and Biological Studies of Aryl Monothiosemicarbazone Palladium(II) Complexes,* presented at INORGANIC 2009, Bloemfontein, South Africa, 2009.

List of Abbreviations and Symbols

tert.	Tertiary
%	Percent
°C	Degree Celsius
5-FU	5-Fluorouracil
Å	Ångstrom (XRD)
ART	Artemisinin
BCNU	1,3-Bis(2-chloroethyl)-*l*-nitrosourea
^{t}Bu	Tertiary butyl
$CDCl_3$	Deuterated chloroform
cm^{-1}	Reciprocal centimeters (IR)
CQ	Chloroquine
d	Doublet (NMR)
DCM	Dichloromethane
dd	Doublet of doublets (NMR)
Decomp.	Decomposition
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
EA	Elemental Analysis
ESI	Electron Spray Ionisation
GC	Gas Chromatography
Hb	Haemoglobin
Hz	Hertz (NMR)
IC_{50}	50 % inhibition *in vitro*
IR	Infrared
J	Coupling constant (NMR)
kDa	Kilodaltons
lit.	Literature

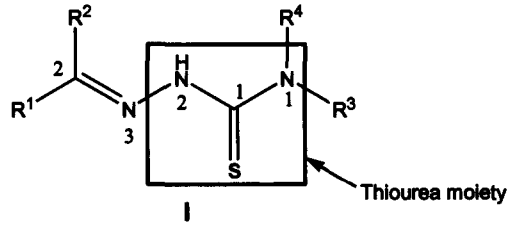
m	Multiplet (NMR); medium intensity (IR)
m.p.	Melting point
m/z	Mass-Charge ratio (MS)
Me	Methyl
MHz	Megahertz, NMR
MS	Mass Spectrometry
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NMR	Nuclear Magnetic Resonance
OMe	Methoxy
PARP	Poly Adenosine-Diphosphate Ribose Polymerase
PPh_3	Triphenylphosphine
ppm	Parts per million (NMR)
q	Quartet (NMR)
s	Singlet (NMR); strong intensity (IR)
t	Triplet (NMR)
TLC	Thin layer chromatography
TMS	Trimethylsilane
TON	Turnover number
w	Weak intensity (IR)
XRD	X-ray diffraction

Table of Contents

Chapter 1: General Survey of Tridentate Monothiosemicarbazone Palladium(II) Complexes

1.1. Brief Background on Thiosemicarbazones

Thiosemicarbazones are Schiff-base type compounds that are noted for their pharmacological properties, particularly as antiparasitic,[1-6] antibacterial [7-9] and antitumoral agents.[10-14] These compounds contain a thiourea moiety which has several donor atoms (Figure 1.1.) and are capable of acting as multidentate ligands toward a metal.



I

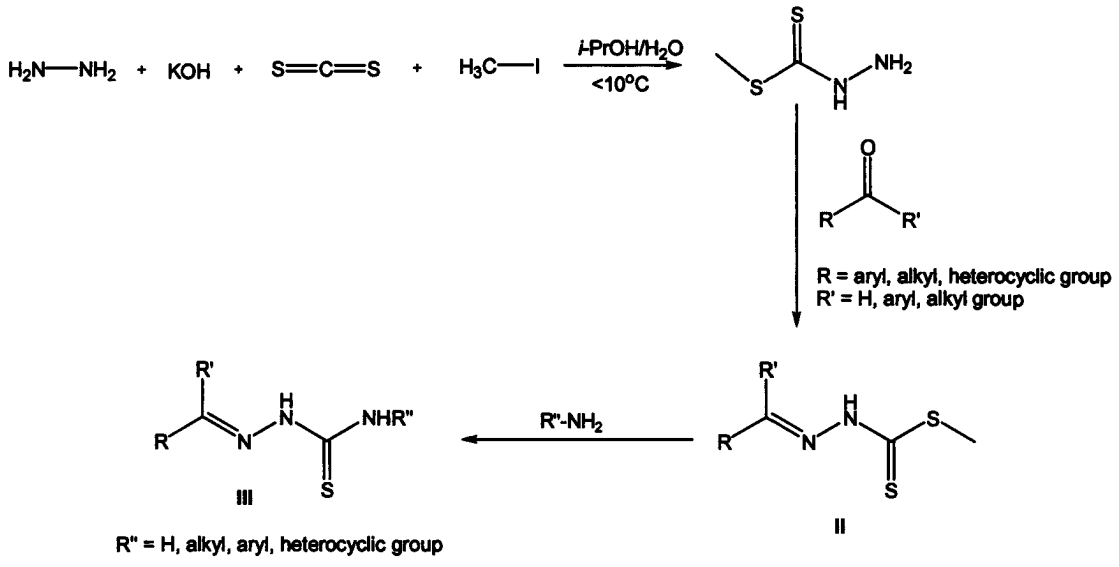
R^1 = aryl, alkyl, heterocyclic group
R^2 = H, aryl, alkyl group
R^3 = H, alkyl, aryl, heterocyclic group
R^4 = H, alkyl, aryl, heterocyclic group

Figure 1.1: General structure of thiosemicarbazones

In addition to the thiourea moiety, varying the R substituents gives rise to a wide range of compounds.[15] For example, thiosemicarbazones derived from aldehydes have a hydrogen atom (R^2) on the C_2 carbon and the R^1 substituent may be an alkyl, aryl or heterocyclic group. Ketone-derived thiosemicarbazones can have an aryl or alkyl group as the R^2 substituent and in some cases the ketone used is cyclic leading to the C_2 carbon being part of a ring. The substituents (R^3 and R^4) on the N_1 nitrogen atom can be two hydrogens or hydrogen and an alkyl, aryl or heterocyclic group.

While several synthetic routes for the preparation of thiosemicarbazones have been published,[16-22] there are three general routes used to synthesize thiosemicarbazones. The first route involves the preparation of methyl

hydrazinecarbodithioate (Scheme 1.1.).[16,22] This precursor is then condensed with different ketones or aldehydes to give a Schiff base type methyl hydrazine carbodithioate precursor (**II**). Amination of **II** yields the desired thiosemicarbazone (**III**).

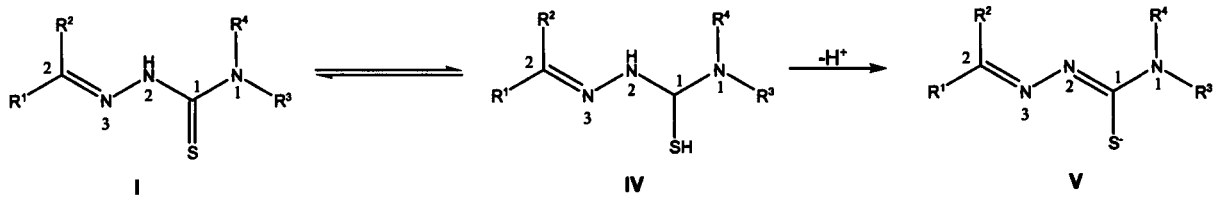


Scheme 1.1.

The second route involves the condensation of a hydrazine derivative with an isothiocyanate to give the desired thiosemicarbazone and the third route, by far the most common method of preparation, yields the thiosemicarbazone by Schiff base condensation of an aldehyde or ketone with thiosemicarbazide.[1,16]

1.2. Bonding Modes of Monothiosemicarbazones

Monothiosemicarbazone ligands exist as an equilibrium mixture of the thione (**I**, Figure 1.2.) and thiol (**IV**) tautomers. Tautomer **I** can bind to a metal as a neutral ligand and tautomer **IV** can bond to a metal as an anionic ligand upon loss of the thiol proton (**V**).[23]



I IV V

Figure 1.2.: Structures of tautomers **I**, **IV** and anionic **V**

Several bonding modes have been observed for both the neutral and anionic forms of monothiosemicarbazones. In the neutral form, these ligands can bind via the S atom only (**VI**,[24,25] Figure 1.3.), with the sulfur bridging two metal centers (**VII**),[24,26] the imine nitrogen and thione sulfur chelating one metal center (**VIII**) [27,28] or with imine nitrogen and sulfur chelation to one metal center and sulfur bridging a second metal center (**IX**).[28] In addition to these bonding modes, if the substituent at the imine carbon contains a donor atom, three other bonding modes are possible; tridentate coordination of donor atom, imine nitrogen and sulfur to metal center (**X**),[29] tridentate coordination as in **X** and the sulfur bridging (**XI**) [30] or the donor atom bridging (**XII**) [31] another metal center.

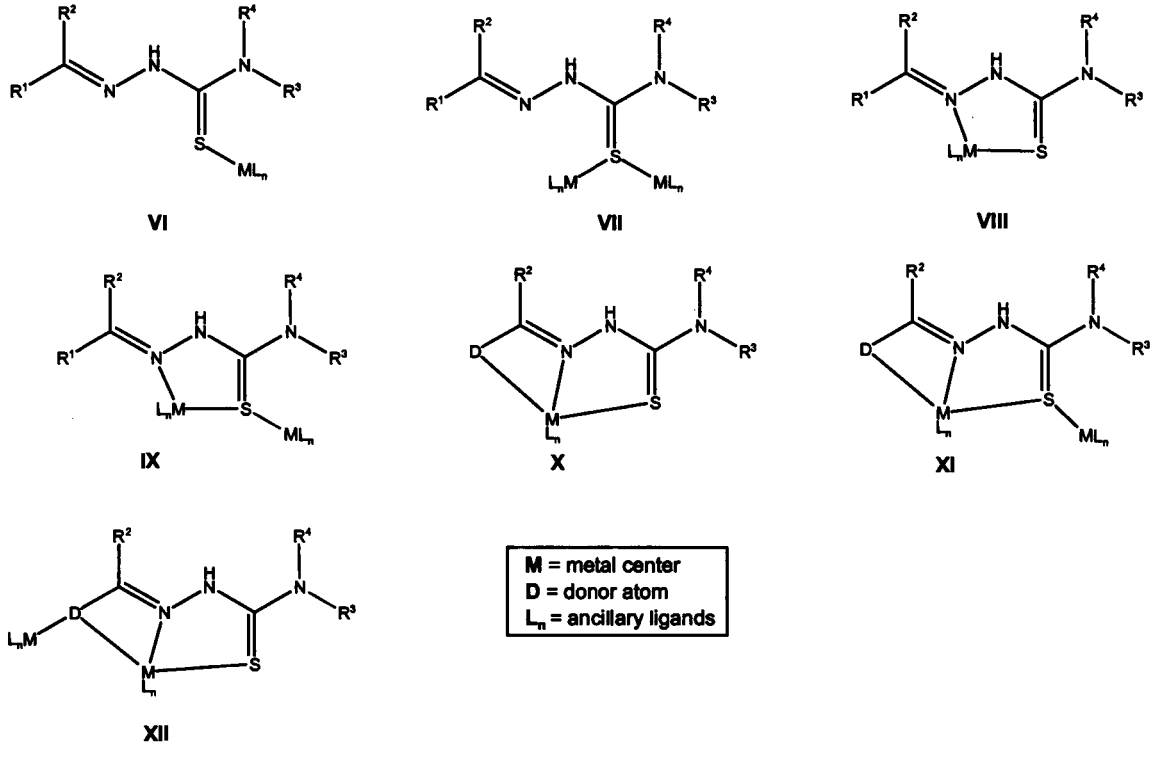


Figure 1.3.: Various bonding modes of thiosemicarbazones in the neutral form (**I**)

For the anionic form **V**, the same bonding modes are observed (with sulfur in the anionic thiolate form) [30-38] as well as bis-chelation of the sulfur and the hydrazinic nitrogen to a metal center (**XIII**,[39] Figure 1.4.) or the sulfur bridging two metal centers and the hydrazinic nitrogen coordinating to a third metal center (**XIV**).[40]

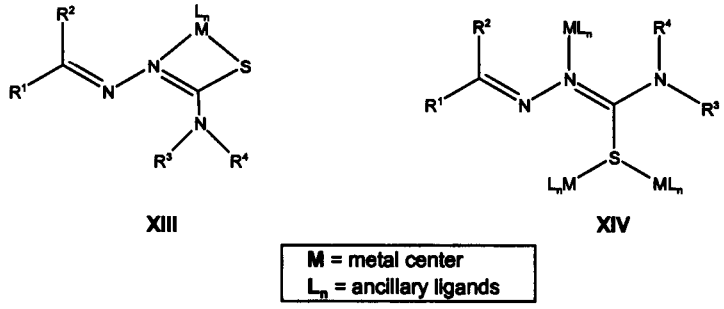


Figure 1.4.: Additional bonding modes of anionic tautomer (**V**)

1.3. Tridentate Aromatic and Heteroaromatic derived Monothiosemicarbazone Palladium(II) Complexes

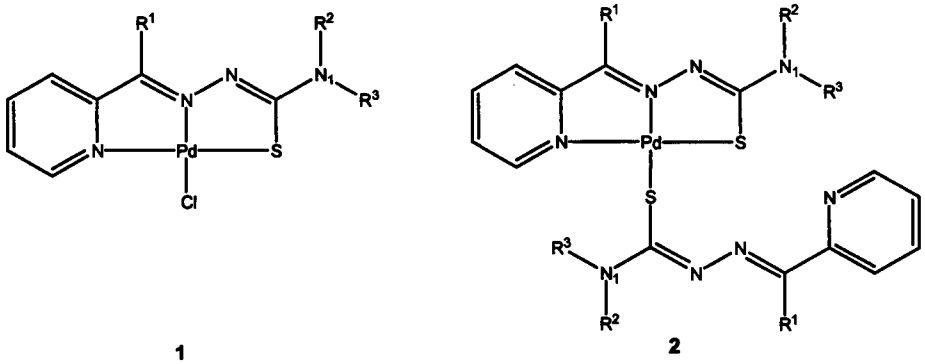
The study of the coordination chemistry of thiosemicarbazones has long been of interest with the earliest review being published in 1974.[41] Since then there have been extensive reports on the synthesis of thiosemicarbazone complexes with metals including vanadium,[42,43] zinc,[44] cobalt,[45] gold,[46] nickel,[47,48] silver,[49] copper,[50-52] and iron.[53] As mentioned in Section 1.1., varying the substituents of the C_2 carbon of thiosemicarbazone ligands influences their bonding mode to the metal. If the substituent contains a donor atom, it is possible for the thiosemicarbazone ligand to bond to palladium in a tridentate manner. Cyclopalladated complexes with tridentate thiosemicarbazones prepared via C-H activation of the C_2 substituent have also been described.[54-56]

Due to the extensive number of literature reports available as well as the relevance of literature examples to this research project, this general survey of the literature focuses on the synthesis of tridentate aromatic- or heteroaromatic-derived monothiosemicarbazone palladium(II) complexes.

1.3.1 Palladium(II) Complexes Containing Tridentate [N,N,S] Donor Monothiosemicarbazones

Literature reports regarding palladium complexes with tridentate [N,N,S] donor thiosemicarbazone ligands mainly comprise thiosemicarbazones derived from pyridine-2-carboxaldehyde or its analogues. Kovala-Demertzi and co-workers have synthesized a variety of these complexes.[10,57-62] When the thiosemicarbazone ligand is reacted with lithium tetrachloropalladate in an approximate 1:1 molar ratio, a chloro palladium complex (**1**) is yielded.[10,58,59,61,62] The ligand coordinates to palladium via the pyridine and imine nitrogen atoms and thiolato sulfur forming two five membered chelate rings and the fourth coordination site on palladium is occupied by a chloride ion.

Palladium(II) complexes of type **2** where two thiosemicarbazone ligands bond to one metal center are isolated when the ligand:metal ratio is approximately 2:1.[10,58,60] One ligand bonds to palladium in the similar tridentate fashion as **1** and a second thiosemicarbazone ligand is bonded to the metal as a monodentate donor via the thiolato sulfur.

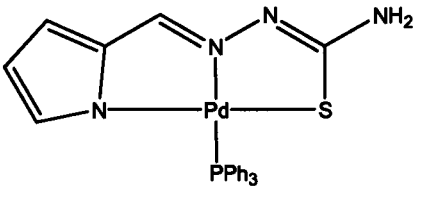


1a and 2a: R^1 = H; R^2 = R^3 = H
1b and 2b: R^1 = Me; R^2 = H, R^3 = Et
1c: R^1 = H; N_1 = hexamethyleneimine ring
1d and 2c: R^1 = Me; N_1 = hexamethyleneimine ring
1e: R^1 = Ph; R^1 = Me, R^2 = Ph
1f: R^1 = H; N_1 = 1-(2-pyridyl)-piperizanyl
1g: R^1 = Me; N_1 = 1-(2-pyridyl)-piperizanyl
1h: R^1 = Me; R^1 = H, R^2 = Et

Figure 1.5. Structures of complexes of type **1** and **2**

The formation of palladium(II) complexes of type **2**, where the ligands form a tridentate/monodentate system instead of a bis-bidentate chelate system are governed by several factors. The preference of palladium to bind to sulfur over nitrogen and its requirement to be as close to square-planar geometry as possible may play a role as well as the need of the ligands to preserve their planar, conjugated structures as far as possible. In addition, the tricyclic ring system comprising the pyridine ring, N-Pd-N chelate ring and N-Pd-S chelate ring of the tridentate ligand would have higher stability compared to a bis-bidentate chelate system.[10]

A mononuclear complex of type Pd(η^3-N,N,S-thiosemicarbazone)(PPh_3) (**3,** Figure 1.6.) was isolated when pyrrole-2-carbaldehyde thiosemicarbazone was reacted with the precursor, $Pd(PPh_3)_2Cl_2$, in the presence of a base.[37] A ligand to metal ratio of 1:1 or 1:2 invariably gave complex **3** upon deprotonation of the pyrrole and hydrazinic nitrogens. When this method was extended to pyridine-2-carbaldehyde, a chloro palladium complex of type **1** was isolated.

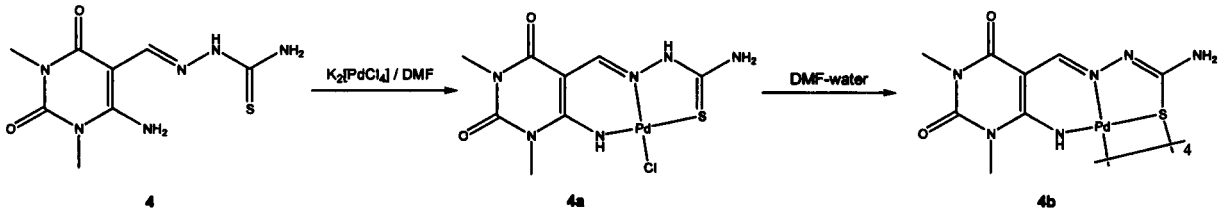


3

Figure 1.6. Structure of complex **3**

The difference in ancillary ligand occupying the fourth coordination site on palladium, despite the same synthetic method being employed, was reasoned in terms of the presence of an acidic hydrogen. Deprotonation of the pyrrole nitrogen facilitates coordination of the thiosemicarbazone as a dianionic ligand thus retaining one triphenylphosphine. The absence of an acidic hydrogen on the pyridine nitrogen means that the ligand bonds to palladium as a singly negative tridentate ligand favouring retention of a chloride ion and loss of triphenylphosphine.[37]

The synthesis of a tetranuclear palladium(II) complex with 6-amino-5-formyl-1,3-dimethyluracil thiosemicarbazone tridentate ligand (Scheme 1.2.) has also been published.[63] Reaction of the ligand (**4**) with potassium tetrachloropalladate in equimolar amounts in DMF gave a mononuclear complex with sulfur coordinating in the thione form (**4a**). However, recrystallisation of this complex from DMF-water solutions yielded the tetrameric complex (**4b**) containing a Pd_4S_4 core that is formed by palladium-sulfur bridging bonds and sulfur is now coordinated to the metal in the thiolato form.

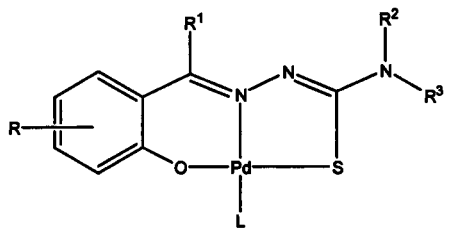


4 4a 4b

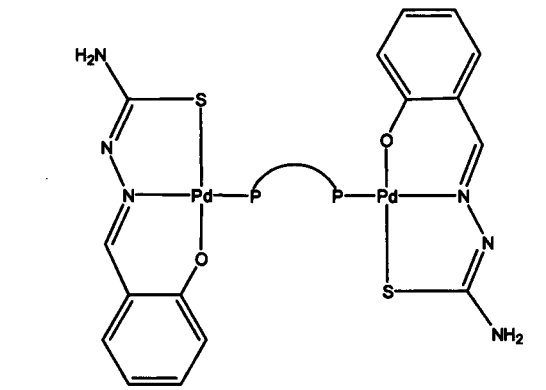
Scheme 1.2.

1.3.2 Palladium(II) Complexes Containing Tridentate [O,N,S] Donor Monothiosemicarbazones

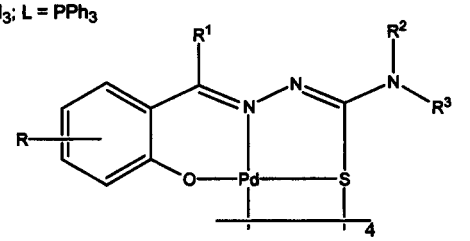
Mononuclear palladium complexes, of type **5** (Figure 1.7.), containing tridentate [O,N,S] thiosemicarbazone ligands have been synthesized by reaction of 2-hydroxy aryl thiosemicarbazone derivatives with a $[Pd(L)_2Cl_2]$ (where L = PPh_3 or 4-picoline) precursor in the presence of a base.[23,37,64,65] In all of the complexes, the ligand forms a six and five membered chelate ring with palladium upon loss of the phenolic and hydrazinic protons. Triphenylphosphine or 4-picoline occupies the fourth coordination site on palladium and the metal has an approximately square-planar geometry. Binuclear complexes (**6**) formed by bridging diphenylphosphinoalkanes have recently been reported.[66] These complexes were prepared using a similar method as for complexes of type **5**.



5a: R, R^1, R^2, R^3 = H; L = PPh_3
5b: R, R^1, R^2, R^3 = H, L = 4-picoline
5c: R, R^2, R^3 = H, R^1 = CH_3; L = PPh_3
5d: R, R^2, R^3 = H, R^1 = CH_3; L = 4-picoline
5e: R, R^1, R^2 = H, R^3 = C_6H_5; L = PPh_3
5f: R = C_4H_4, R^1, R^2, R^3 = H; L = PPh_3
5g: R = C_4H_4, R^1, R^2, R^3 = H; L = 4-picoline
5e: R = C_4H_4, R^1 = R^2 = H, R^3 = C_6H_5; L = PPh_3
5f: R = C_4H_4, R^1 = R^2 = H, R^3 = CH_3; L = PPh_3



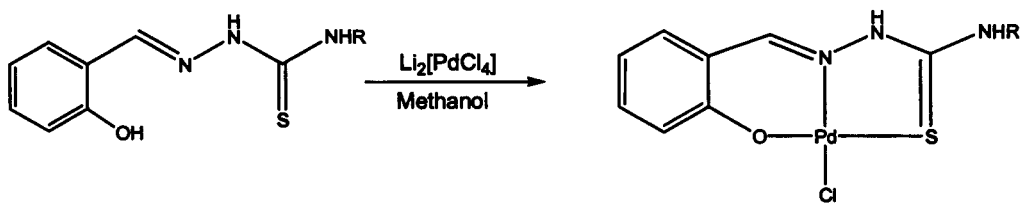
P P = diphenylphoshinomethane (**6a**); diphenylphoshinopropane (**6b**)



7a: R = R^1 = H or CH_3, R^2 = R^3 = H
7b: R = C_4H_4, R^1 = H or CH_3, R^2 = R^3 = H

Figure 1.7. Structure of mononuclear, binuclear and tetranuclear palladium(II) complexes containing tridentate salicylaldehyde thiosemicarbazone derivatives

Tetranuclear complexes (**7**) have been prepared by reaction of the salicylaldimine-thiosemicarbazone with sodium tetrachloropalladate using triethylamine as proton abstractor.[64] In complexes **5** – **7**, the ligand acts as a dianionic tridentate donor toward palladium. If the ligand is reacted with a palladate salt without the use of a base then it coordinates to palladium as a uninegative tridentate donor to the metal (**8**) with sulfur bonding as a neutral donor (Scheme 1.3.).[67]



8a: R = H
8b: R = CH_3

Scheme 1.3.

Palladium(II) complexes of 2-hydroxy-acetophenone thiosemicarbazone analogues have been prepared by reaction of the ligand with a palladate salt. It was demonstrated that changes in pH and stoichiometric ratios affect the type of complex isolated (Figure 1.8.). When a metal:ligand ratio of 1:1 is used at approximately pH 4, mononuclear complexes of type **9** were obtained.[68,69] When the pH of the reaction mixture was raised to approximately 9 using aqueous ammonia, the favoured product was the trinuclear complex of type **10**.[38,68,69] A metal:ligand ratio of 1:2 reacted at pH 9 yielded complex **11**, where one ligand is coordinated as a dianionic tridentate donor to palladium and the second thiosemicarbazone ligand is coordinated in a monodentate fashion via the thiolato sulfur.[70]

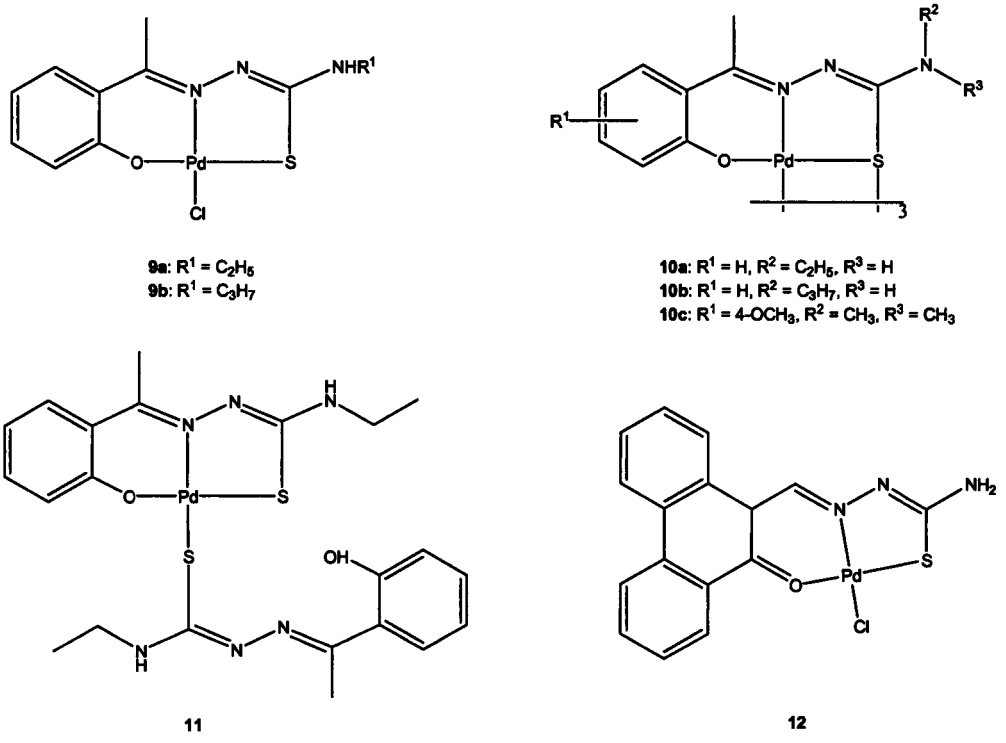
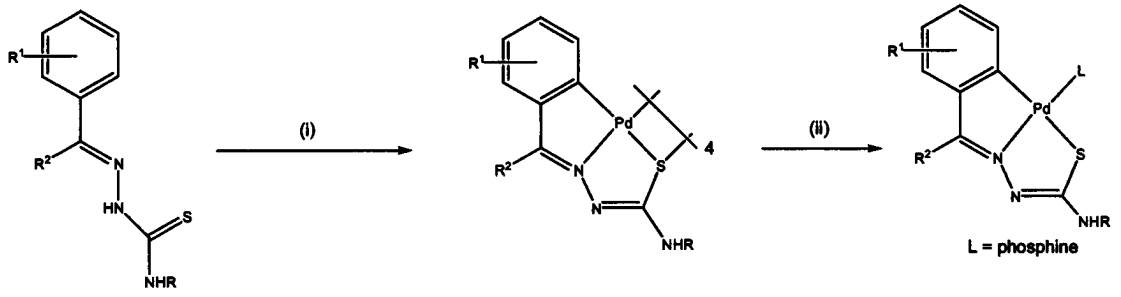


Figure 1.8. Structures of complexes **9 - 12**

The reaction of phenanthrenequinone thiosemicarbazone with potassium tetrachloropalladate yields a mononuclear complex (**12**) where the thiosemicarbazone ligand coordinates to palladium as a uninegative [O,N,S] donor via the carbonyl oxygen, imine nitrogen and thiolato sulfur.[71]

1.3.3 Palladium(II) Complexes Containing Tridentate [C,N,S] Donor Monothiosemicarbazones

The preparation of cyclopalladated aryl thiosemicarbazone complexes can be achieved by treatment of an aryl thiosemicarbazone with a tetrachloropalladate salt or palladium acetate (Scheme 1.4.).[54,55,72,73] The product is a tetrameric complex (**14**) with the ligand bonding as a tridentate [C,N,S] donor to palladium forming two fused rings at the metal center and a Pd_4S_4 core that is formed by Pd-$S_{bridging}$ bonds.



13: R^1, R^2, R = alkyl or H **14** **15**

(i): $Pd(OAc)_2$ or tetrachloropalladate salt; (ii): tertiary phosphine

Scheme 1.4.

The palladium-sulfur bridging bond can be cleaved with a nucleophile such as a tertiary phosphine, yielding a mononuclear *ortho*-palladated complex (**15**). The palladium-sulfur chelating σ-bond remains intact and this can be reasoned in terms of Pearson's concept.[74] Palladium is a borderline/soft acid and will prefer to bond to borderline or soft bases. Sulfur is a soft base and thus, the palladium-sulfur chelating bond in thiosemicarbazone complexes is much stronger than in its corresponding [C,N,O] and [C,N,N] complexes where the metal-oxygen and metal-nitrogen bonds are easily cleaved with a tertiary phosphine leading to opening of the chelate ring since oxygen and nitrogen are harder bases than sulfur.[54]

Binuclear tridentate thiosemicarbazone complexes have been prepared from the tetranuclear complexes by Vila *et al.*[72] They cleaved the Pd-$S_{bridging}$ bond in the tetrameric complex with a variety of tertiary diphosphines including

bis(diphenylphosphino)ferrocene using a 1:2 molar ratio of complex to diphosphine (Figure 1.9.). In these binuclear complexes (**16**), one thiosemicarbazone ligand is coordinated in a dianionic [C,N,S] manner to each metal center. The two palladium metal centers are bridged by the bis(diphenylphosphino) ligand. Mononuclear complexes (**17**) are isolated when a 1:4 stoichiometric ratio is used.[75] The diphosphine acts as a monodentate ligand via one phosphorus atom.

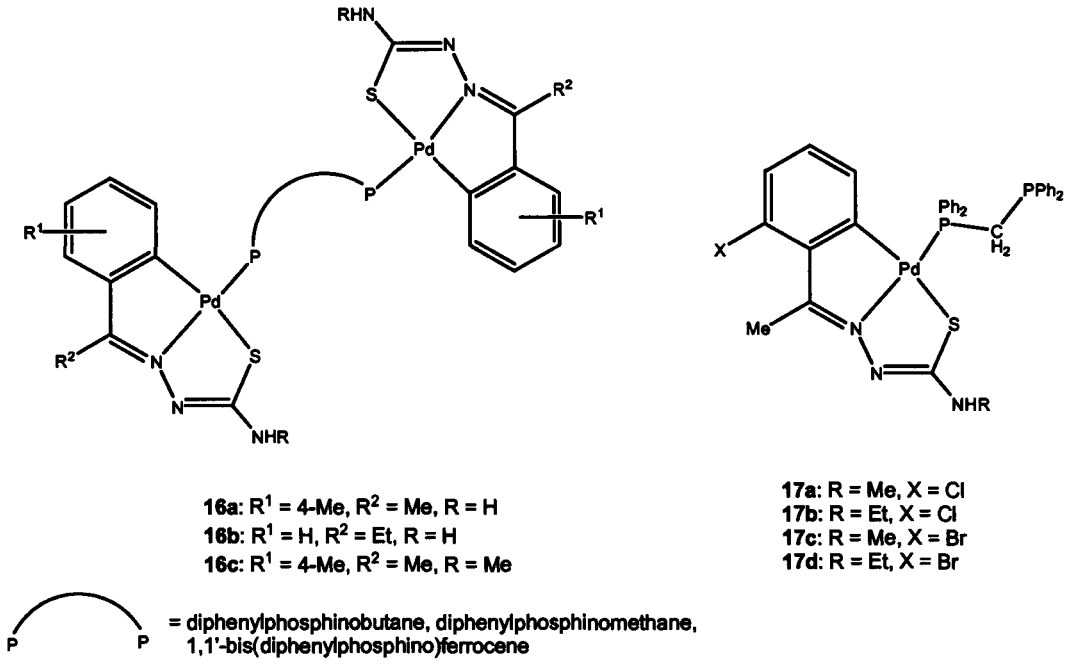


Figure 1.8. Structures of binuclear complexes (**16**) formed by bridging diphenylphosphino ligands and mononuclear complexes (**17**) with monodentate diphoshine ligand

Mononuclear cyclopalladated thiosemicarbazone complexes with a chloride ion as ancillary ligand have been directly prepared by reaction of the thiosemicarbazone ligand with potassium tetrachloropalladate (**18**, Figure 1.9.).[55]

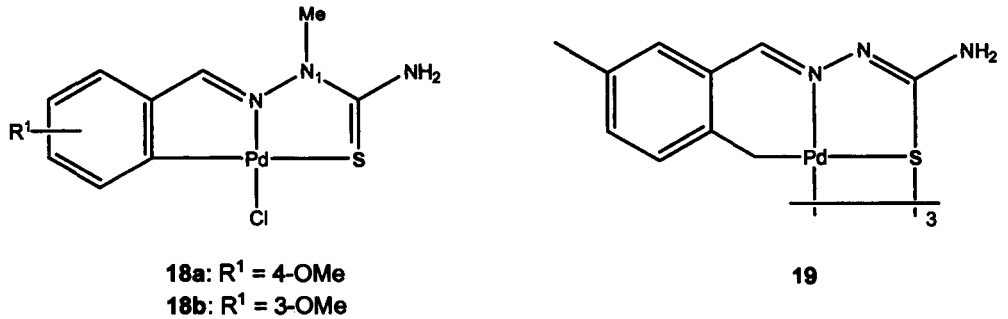


Figure 1.9. Structures of mononuclear (**18**) and trinuclear (**19**) cyclopalladated complexes

In the thiosemicarbazone ligand, the N_1 nitrogen is substituted with a methyl substituent blocking formation of the thiolate which is essential for formation of the tetranuclear complex of type **14**. The ligand therefore chelates to palladium as a singly negative tridentate [C,N,S] donor. A trinuclear palladium(II) complex (**19**) prepared by C-H activation of a sp^3 carbon has also been accomplished.[76] The imine nitrogen and the carbon of the methyl substituent in the *ortho* position coordinate to palladium resulting in the formation of a six-membered chelate ring.

1.4. Biological Activity of Tridentate Palladium(II) Monothiosemicarbazone Complexes

The biological properties of thiosemicarbazones may, in part, be due to their metal chelating abilities; complexation to a metal ion within the cell may give rise to the actual active species. In addition, complexation to a metal could beneficially alter the bioactivity of thiosemicarbazones.[22] Lipophilicity, which controls the rate of entry into the cell, might be modified upon coordination.[77] The metal complex may be more active than the free ligand and certain long term side effects may decrease or be altogether avoided since metal complexes can break down and the metal ion can then interact with the organism.[78,79] Furthermore, the metal complex could exhibit bioactivities not shown by the free thiosemicarbazone ligand and coordination can yield a significant reduction in drug resistance.[22]

These reasons, together with the established activity of thiosemicarbazones, have led to an ever-increasing amount of research into the biological application of their metal complexes. For example, metallocene derivatives of titanium and zirconium with thiosemicarbazones as well as oxovanadium(IV) complexes of 2-pyridine carboxaldehyde have shown activity as antifungal agents;[80,81] four and six coordinate Ni(II) thiosemicarbazone complexes have been screened for activity as anti-microbial agents.[82]

1.4.1. Brief Background on Palladium(II) Complexes as Anticancer Agents

The antitumoral activity of cis-[diaminedichloroplatinum(II)] (cisplatin or cis-DDP) led to the investigation of other metal complexes as anticancer agents since cisplatin, while exhibiting good activity, is highly toxic. Neurotoxicity (nerve damage), ototoxicity (hearing loss) and nephrotoxicity (kidney damage) are just some of the side effects induced by cisplatin.[83] Therefore, almost from this initial discovery efforts have been made to attain cisplatin's beneficial properties while reducing its toxicity. The synthesis and antitumor evaluation of a large number of platinum complexes has been accomplished; out of these complexes carboplatin, [diamine(cyclobutane-1,1-carboxylato)platinum(II)], has shown activities equivalent to cisplatin but with a markedly lower toxicity.[84,85]

In the search for more efficient metal complexes relative to cisplatin, palladium(II) complexes were amongst the first to be used in clinical trials for the treatment of tumors since they exhibit similar aqueous chemistry to those of platinum(II) and both metals have similar chemical properties.[86] Platinum and palladium belong to the second transition metal series and the +2 and +4 states are their most stable oxidation states. They are classified as soft Lewis acids, forming stronger bonds with sulfur and nitrogen donors than oxygen.[74] However, palladium(II) complexes are generally thermodynamically and kinetically less stable than Pt(II) complexes; palladium(II) complexes undergo ligand exchange reactions and aquation at a rate 10^5 times faster than the analogous platinum(II) complexes.[83,86,87] This last property explained why the complexes *cis*-$Pd(NH_3)Cl_2$ and $Pd(1,2\text{-diaminocyclohexane})_2Cl_2$ were found to have lower activity and higher toxicity than its platinum analogues.[83]

Since then, interest has moved toward the preparation of Pd(II) complexes with inert ligands such as sulfur donors. It has been suggested that N,S chelates of Pd(II) are better antitumor agents than those of other metals. Nickel(II), copper(II) and zinc(II) N,S chelate complexes do not have sufficient thermodynamic stability while platinum(II) N,S chelates are kinetically inert. In contrast, palladium(II) chelates

possess the proper lability to transport the metal to DNA, allowing it to interact with it.[88]

Thiosemicarbazones have arisen as an excellent class of N,S donor ligands in the search for better antitumoral palladium(II) complexes. These ligands display anticancer activity and it is believed that their mechanism of action is through the inhibition of ribonucleotide reductase, an enzyme that catalyzes the conversion of ribonucleotides to deoxyribonucleotides, by either coordination to iron in the metal-bound enzyme or formation of an iron chelate *in situ* which acts as the active inhibitor of the enzyme.[89] Their use in conjunction with Pd(II), which is known to damage DNA, produces a synergistic effect which could potentially lead to the development of more effective cancer chemotherapies. Thus, the study of Pd(II) thiosemicarbazone complexes as antiproliferative agents has emerged as an area of great importance.

1.4.2. Anticancer Activity of Aromatic and Heteroaromatic Derived Tridentate Monothiosemicarbazone Palladium(II) Complexes

The effects of complexes **1d** and **2c** (Figure 1.10, page 15) and their free ligand on DNA synthesis of P388 and L1210 , mouse lymphocytic leukemia, cell cultures and in leukemia P388 bearing mice have been studied.[90,91] The free ligand was found to exhibit the highest LD_{10}, the dose required to induce death of 10% of mice used, while the complexes exhibited significant reduction of the toxicity, an enhancement of cytogenetic damage and a significant increase in the mean survival of the drug treated leukemia bearing mice.

Thiosemicarbazones derived from 2-benzoyl pyridine (**20a – 20c**) and their tridentate [N,N,S] palladium(II) complexes (**21a – 21c**) were screened for cytotoxic activity against the MCF-7 (human breast cancer), TK-10 (human renal carcinoma) and UACC-62 (human melanoma) cell lines (Table 1.1.).[92]

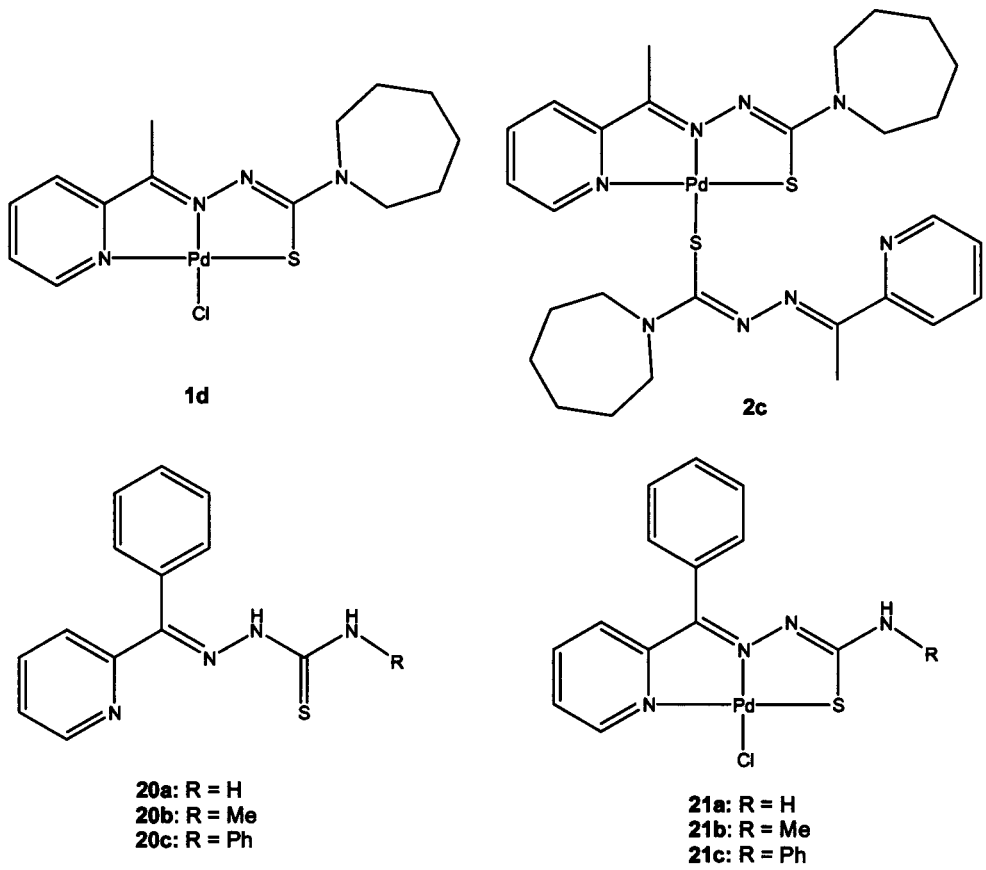


Figure 1.10. Structures of complexes **1d**, **2c**, **20a – 20c** and **21a – 21c**

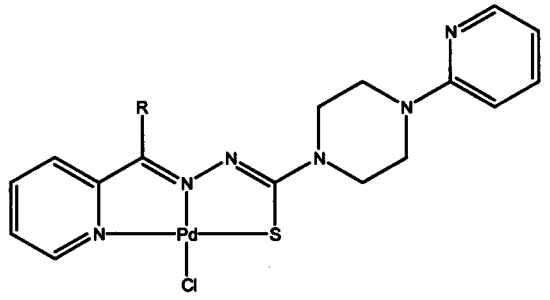
Table 1.1. Compound concentration that produces 50 % of growth inhibition (GI_{50}) and lethal dose (LC_{50}) for 50 % of the cells for compounds **20a – 21c**

	MCF-7		TK-10		UACC-62	
Compound	**GI_{50} (μM)**	**LC_{50} (μM)**	**GI_{50} (μM)**	**LC_{50} (μM)**	**GI_{50} (μM)**	**LC_{50} (μM)**
20a	2.7	>390.3	4.7	>390.3	5.1	93.3
20b	0.0007	>369.9	1.8	>369.9	0.2	25.9
20c	<0.003	13.4	9.3	12.9	<0.003	13.8
21a	75.5	146	120.3	217.8	99.5	133.2
21b	9.5	56.4	27.5	32.6	25	42.6
21c	6.4	63	24.3	29.6	22.2	33

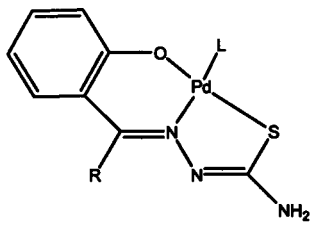
The ligand **20c** showed the best GI_{50} and LC_{50} values against all three cell lines. Complexes **21a – 21c** displayed GI_{50} (50 % growth inhibition) values higher than their free ligands indicating lower cytotoxicity. However, complexes **21b** and **21c** exhibited lower LC_{50} values compared to the free ligands against the TK-10 and MCF-7 cell lines. This indicates that a lower dose of the complex is needed to kill

the cancer cells compared to their corresponding free ligand (**20b** and **20c**) and is suggestive of a different mechanism of inhibition for the complexes relative to the free ligands.

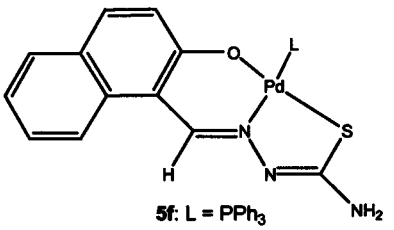
The palladium(II) complexes (**1f** and **1g**) of 2-formyl pyridine thiosemicarbazone derivatives were examined for activity against the MCF-7, T24 (bladder cancer) and A-549 (non-small cell lung carcinoma) cell lines.[61] Complex **1g** (Figure 1.11.) showed better cytotoxicity, IC_{50} = 3.75 μM, compared to **1f** in the MCF-7 cell line which displayed an IC_{50} value of 83.9 μM. In addition, **1f** was found to be twice as active as cisplatin against this cell line. In contrast, both complexes showed similar activities, IC_{50} values of 4.89 and 5 μM for **1f** and **1g** respectively, against the T-24 cell line, which are significantly higher than that of cisplatin for this cell line. Complex **1f** exhibited an IC_{50} = 12.8 μM against the A-549 cell line while **1g** did not show any appreciable activity.



1f: R = H
1g: R = CH_3



5a: R = H, L = PPh_3
5b: R = H, L = 4-picoline
5c: R = CH_3, L = PPh_3
5d: R = CH_3, L = 4-picoline



5f: L = PPh_3
5g: L = 4-picoline

Figure 1.11.: Structures of palladium(II) complexes **1f, 1g , 5a – 5d, 5f** and **5g**

Halder and co-workers reported on the *in vitro* growth inhibitory effects of salicylaldehyde (**5a** and **5b**), 2-hydroxyacetophenone (**5c** and **5d**) and 2-hydroxynaphthaldehyde (**5f** and **5g**) thiosemicarbazone tridentate [O,N,S] palladium(II) complexes in two cancer cell lines promyelocytic HL-60 and histiocytic lymphoma U-937.[64] The results were compared to the human clinical drugs cisplatin, BCNU, 5-FU and hydroxyurea (Table 1.2.).

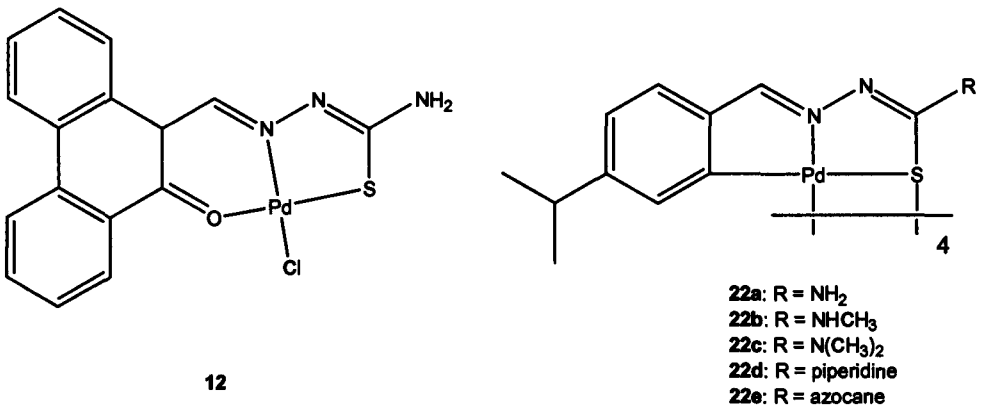
Table 1.2.: IC_{50} values (μM) for **5a-5d**, **5f** and **5g** and selected clinical drugs against the HL-60 and U-937 cell lines

Compound	HL-60	U-937
5a	2.5	4.8
5b	16.2	7.3
5c	0.6	1.3
5d	7.1	6.6
5f	203	231.6
5g	6.5	7.7
Cisplatin	7.0	3.2
BCNU	30.5	12.3
5-FU	266.0	4.7
Hydroxyurea	204.0	115.0

In the HL-60 cell line, compounds **5a** and **5c**, where the ancillary ligand is triphenylphosphine, exhibited better activity than the other complexes as well as the clinically used drugs. With the exception of **5f**, which did not show any appreciable activity in either cell line, all of the complexes show activities less than 10 μM against the U-937 cell line and it should be noted that the complex with the lowest IC_{50} value (**5c**) is a better cell growth inhibitor than cisplatin. Apoptosis experiments were also carried out for complexes **5c** and **5g**. It was found that **5c** induced apoptosis to a greater extent than cisplatin.

The phenanthrenequinone thiosemicarbazone complex (**12**) was evaluated for antiproliferative properties in a panel of breast cancer and normal cell lines.[71] It was found that the complex was a potent inhibitor of cell growth in the MCF-7/DOX cell line which exhibits high resistance against conventional chemotherapeutic agents. It

was less effective against the wild-type MCF-7 cell line suggesting that it could be used beneficially in the treatment of the resistant tumor cells. The normal cell lines were relatively resistant to the toxic effects of complex **12** signifying that its growth inhibitory effects are specific to tumor cell lines.



12

Figure 1.12.: Structure of complex **12** and tetrameric cyclopalladated *p*-isopropylbenzaldehyde thiosemicarbazone derivatives (**22a – 22e**)

The cytotoxic activity of tetranuclear cyclopalladated derivatives of *p*-isopropylbenzaldehyde has been reported by Quiroga *et al.*[93-95] The complexes (**22a – 22e**) were screened against the HL-60, JURKAT (human leukemia), HeLa (cervix epithelial carcinoma), 3T3 (murine fibroblasts), Pam 212 (epidermal keratinocyte) and Pam 212-*ras* (epidermal keratinocyte cells transformed by H-*ras* oncogene) cell lines and their IC_{50} values are shown in Table 1.3.

Table 1.3.: IC_{50} and standard deviation values (μM) for complexes **22a – 22e**

	HL-60	JURKAT	HeLa	3T3	Pam 212-*ras*	Pam 212
22a	28 ± 1	7 ± 0.3	4 ± 0.1	3 ± 0.2	5 ± 0.4	8 ± 0.3
22b	22 ± 2	13 ± 1	86 ± 3	91 ± 5	62 ± 2	109 ± 6
22c	29 ± 1	25 ± 3	92 ± 3	107 ± 4	96 ± 7	134 ± 7
22d	10 ± 0.7	9 ± 1	73 ± 3	94 ± 1	68 ± 2	90 ± 3
22e	27 ± 2	18 ± 0.7	87 ± 3	104 ± 2	76 ± 2	164 ± 0.8

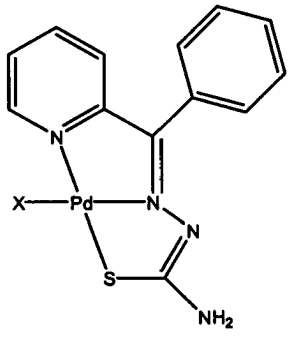
The complexes **22a – 22e** exhibited activities in the μM range against all cell lines. However, all of them were less active than cisplatin against the HL-60 cell line. The complex **22a** exhibited better cytotoxicity than cisplatin across all cell lines, with the

exception of the HL-60 cell line. It was also noted that while the complexes **22b** – **22e** did not show appreciable activity overall, their cytotoxic values were better than those shown by cisplatin and other clinically used drugs against the JURKAT cell line. The Pam 212 and Pam 212-*ras* cell lines are cisplatin resistant and complexes **22a**, **22b** and **22d** show moderate activity against Pam 212-*ras* cells.

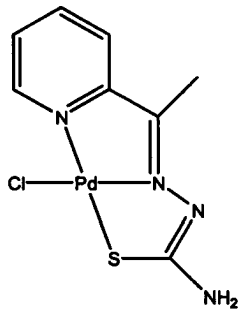
1.5. Application of Palladium(II) Thiosemicarbazone complexes as catalysts or catalyst precursors

Palladium catalyzed reactions have become essential synthetic tools; their applications including the syntheses of polymers,[96,97] natural products,[98] agrochemicals[99,100] and pharmaceuticals.[101,102] There are several well-known reactions that utilize palladium containing compounds including the Heck, Stille, Suzuki and Buchwald-Hartwig cross-couplings.[103,104] In addition, palladium has also been used to facilitate hydrogenation, carbonylation, cycloisomerization and even pericyclic reactions.[103]

The application of thiosemicarbazone palladium(II) complexes in catalysis is a fairly new area of research. One of the first reports of their catalytic application was published in 1998. Pelagatti and co-workers described chemoselective homogenous hydrogenation reactions using tridentate [N,N,S] palladium(II) complexes **21a, 23a, 23b** and **24** (Figure 1.13).[105]



21a: X = Cl
23a: X = CH_3CO_2
23b: X = I



24

Figure 1.13.: Structures of complexes **21a, 23a, 23b** and **24** used for chemoselective homogenous hydrogenation

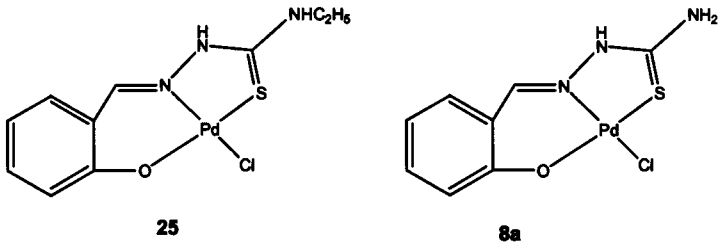
The reactions were carried out at 30°C under atmospheric pressure of hydrogen over 24 hours with DMF, pyridine or methanol as solvent. Table 1.4. shows the hydrogenation data. When the reaction solvent was methanol, the iodo-complex **23b** was unable to hydrogenate phenylacetylene, complexes **21a** and **23a** showed good catalytic activity. Overall, **21a** showed the best activity in methanol; it was able to hydrogenate phenylacetylene completely with good selectivity toward styrene. When **21a** was used with DMF or pyridine as solvent its catalytic activity was lower. For complex **24** the best results were observed when the reaction was carried out in DMF.

Table 1.4.: Hydrogenation data for phenylacetylene using complexes **21a**, **23a**, **23b** and **24** as catalysts[a,b]

Complex	Solvent	Phenylacetylene (%)	Styrene (%)	Ethylbenzene (%)
21a	methanol	-	92	8
23a	methanol	-	44	56
23b	methanol	100	-	-
21a	dmf	61	37	2
24	dmf	-	95	5
21a	pyridine	70	30	-
24	pyridine	75	23	2

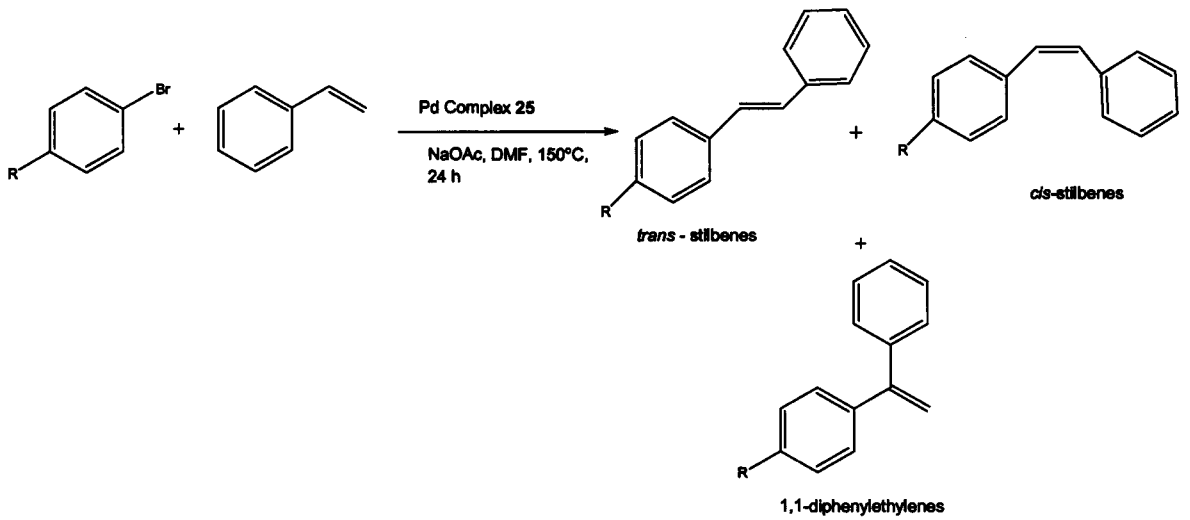
[a] Catalytic reactions were carried out at 30°C and 1 atm of hydrogen for 24 hours.
[b] [phenylacetylene] = 1.44×10^{-1} mol.dm^{-3} and [cat] = 1.44×10^{-3} mol.dm^{-3}

The palladium(II) complex (**25**) containing a tridentate uninegative [O,N,S] donor thiosemicarbazone (Figure 1.14.) was evaluated as a catalyst precursor for the Heck reaction of styrene with electron-rich and electron-poor 4-substituted aryl bromides (Scheme 1.5.).[106] The reactions were carried out in DMF at 150°C for 24 hours using sodium acetate as base under an inert atmosphere and in air. Table 1.5. summarizes the results of the catalytic studies. High regioselectivity was observed for the formation of *trans*-stilbenes and the presence of *cis*-stilbenes or 1,1-diphenylethylenes was either absent or only observed in trace amounts.



25 8a

Figure 1.14.: Structures of complexes **8a** and **25**



Scheme 1.5.

Table 1.5.: Heck Reaction of aryl bromides with styrene catalyzed by complex **25**

R	Atmosphere	ArBr/Pd ratio	GC Yield (%)	TON
OMe	argon	1000	46	460
OMe	air	1000	12	120
OMe	argon	100000	17	17000
H	argon	1000	54	540
H	air	1000	18	180
H	argon	100000	18	18000
CHO	argon	1000	95	950
CHO	air	1000	94	940
CHO	argon	100000	30	30000
CHO	air	100000	14	14000
NO_2	argon	1000	95	950
NO_2	air	1000	94	940
NO_2	argon	100000	43	43000

The catalytic activity depended on the halide substrate, the highest activity was observed for the reaction where R = NO_2. Under argon, a total yield of 46% was seen for the highly inactive 4-bromoanisole when the catalyst:substrate ratio was 1:1000. Only a yield of 12% was observed for the same catalyst:substate ratio in air. For the activated aryl bromides, similar yields were seen in air and under argon when the ratio of catalyst:substrate was 1:1000. A yield of 18% was observed for 4-bromobenzaldehyde at very low concentrations of palladium and also when the reaction was carried out in air. Overall, while some catalytic activity was exhibited by **25** when the reaction is carried out in air, better activities were seen under an inert atmosphere. This is possibly due to the fact that **25** is thermally stable under argon.[106]

The catalytic activity of **25** together with its analog **8a** was then assessed for the Suzuki-Miyaura cross-coupling reaction of aryl bromides and chlorides with phenyl-boronic acid under aerobic conditions.[107] Table 1.6. summarizes the results of the study.

Table 1.6.: Percent conversion of the Suzuki-Miyaura cross-coupling reactions of aryl halides with phenyl boronic acid catalyzed by complexes **25** and **8a**

Catalyst	ArX (X; R)	ArX:Pd ratio	% conversion
25	Br; OMe	1000	40
8a	Br; OMe	1000	53
25	Br; H	1000	71
8a	Br; H	1000	51
8a	Br; H	1000	71
25	Br; CN	1000	78
8a	Br; CN	1000	88
25	Br; CN	100000	13
8a	Br; CN	100000	39
25	Br; NO_2	1000	79
8a	Br; NO_2	1000	83
25	Br; NO_2	100000	31
8a	Br; NO_2	100000	49
25	Cl, H	1000	30
8a	Cl, H	1000	26
25	Cl; NO_2	1000	36
8a	Cl; NO_2	1000	37

A stock solution of **25** and **8a** in DMF was used at 100°C over 24 hours with Na_2CO_3 as the base. All the reactions were performed in air and the addition of one equivalent of water with respect to the substrates enhanced the catalytic activity. It was noted that the catalysts were air stable at 100°C and the deposition of palladium black was not observed. Also, the homocoupling of phenylboronic acid to give unsubstituted biphenyl was negligible.[107]

Conversions ranging from 40 to 71 % were seen for 4-bromoanisole and bromobenzene. Conversions of 80 % or higher were observed for 1-bromo-4-nitrobenzene and 4-bromobenzonitrile. For the cross-coupling reactions where an aryl chloride was the substrate low conversions were observed. This could be due to the high C-Cl bond strength which makes oxidative addition of the substrate across the metal centre difficult.[107]

1.6. General Conclusions

Thiosemicarbazones have emerged as a class of versatile ligands due to their ability to adopt several different bonding modes toward a metal. Their established biological acitivities has led to a growing interest in the chemistry and application of their transition metal complexes. It is important to note that while reports on the use of palladium complexes of aryl-derived monothiosemicarbazone complexes as biological agents is growing; the study of their tridentate palladium(II) complexes for anticancer activity is still relatively scarce.

In addition, the application of palladium thiosemicarbazone complexes as catalysts or catalyst precursors is still virtually unexplored. From the few reports available their ability to catalyze Suzuki-Miyaura coupling reactions in the presence of water and in air shows their potential as green catalysts due to their high thermal, air and moisture stability and warrants further investigation.

1.7. Aims and Objectives of Project

1.7.1. *Aims:*

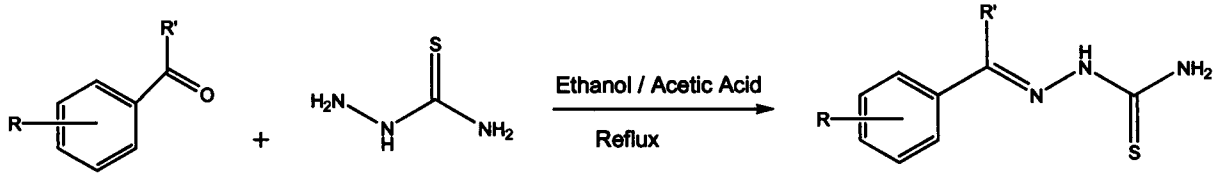
The overall objective was to prepare a series of palladium(II) complexes with tridentate thiosemicarbazones and to study their activity as

(i) antimalarial / anticancer agents
(ii) catalyst precursors in cross coupling reactions

1.7.2. Specific Objectives:

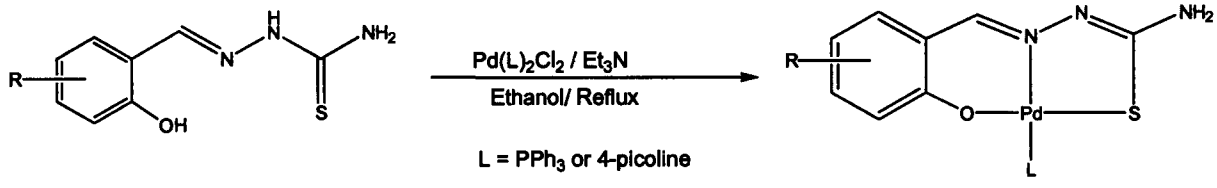
The specific objectives of the research described in this dissertation are:

- To synthesize and characterize a series of aryl mono-thiosemicarbazone ligands as outlined in Scheme 1.6.

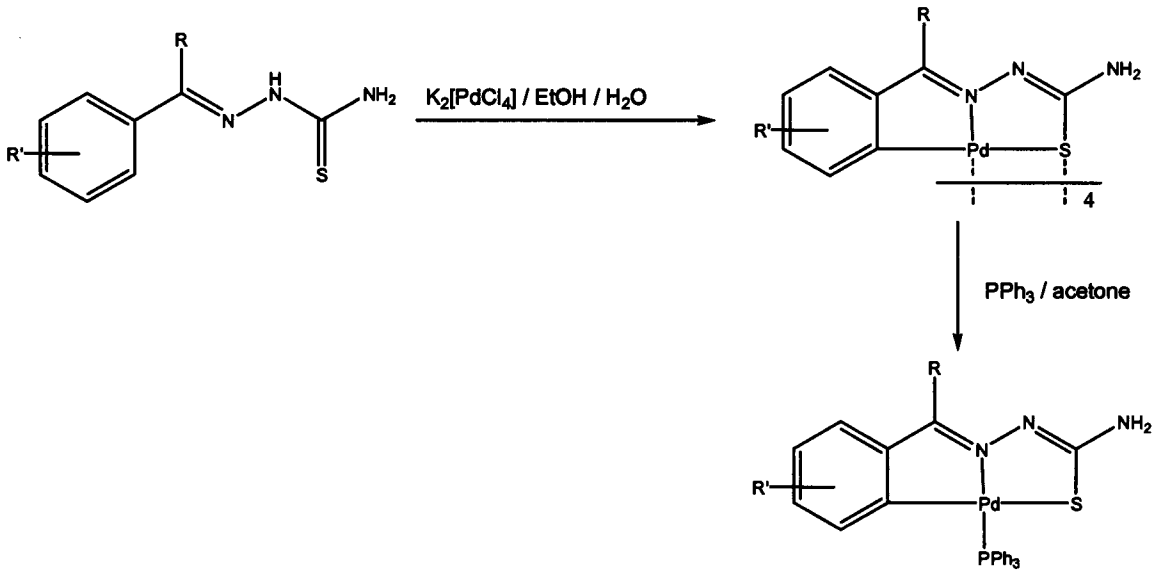


Scheme 1.6.

- To synthesize tridentate [O,N,S] and [C,N,S] palladium(II) complexes of the thiosemicarbazone ligands synthesized according to Schemes 1.7. and 1.8.



Scheme 1.7.



Scheme 1.8.

- To evaluate selected Pd(II) complexes and their corresponding free ligands *in vitro* for anticancer activity against several cell lines and antimalarial activity against the *P. falciparum* strains, W2 (chloroquine-resistant) and D10 (chloroquine- sensitive).

- To evaluate the potential activity of selected Pd(II) complexes as catalyst precursors in the Suzuki-Miyaura cross-coupling reaction.

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Chapter 2: Synthesis and Characterization of Tridentate [O,N,S] Monothiosemicarbazone Palladium(II) Complexes

2.1. Introduction

Thiosemicarbazones have been shown to exhibit several types of bonding modes, as discussed in length in Chapter 1. The most common type of bonding mode for salicylaldehyde derived thiosemicarbazones is as tridentate [O,N,S] donors.[1-5] There have been extensive reports of the complexation of salicylaldehyde thiosemicarbazone to different metals including, ruthenium,[1] nickel,[2] cadmium,[6] mercury,[6] platinum,[7] cobalt,[8] silver [9] and copper.[4]

Mononuclear palladium complexes (**5**, Figure 2.1) can be directly prepared by reaction of the ligand with $[Pd(L)_2Cl_2]$ (where L = PPh_3 or 4-picoline) in refluxing ethanol.[5,10-12] The tetranuclear complex (**7a**) can also be prepared by reaction of the salicylaldehyde thiosemicarbazone with sodium tetrachloropalladate[10]. A trimeric Pd(II) complex of 2-hydroxyacetophenone (**10d**) has also been reported.[13] In all of these complexes, the ligand bonds to palladium as a dianionic [O,N,S] chelate via the thiolate sulfur and the deprotonated phenolic oxygen.

Coordination of the salicylaldimine thiosemicarbazone ligand to palladium in the thione conformation is also possible.[14] Reaction of the ligand with lithium tetrachloropalladate in methanol without the use of a base produces a mononuclear chloro-palladium complex (**8**) where the ligand coordinates as an anionic tridentate donor.

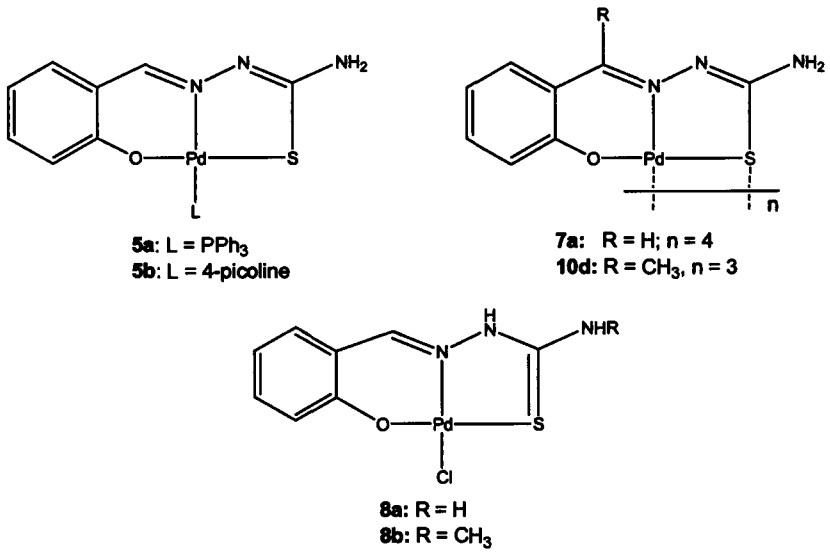


Figure 2.1. Representative structures of certain salicylaldimine thiosemicarbazone palladium(II) complexes previously synthesized

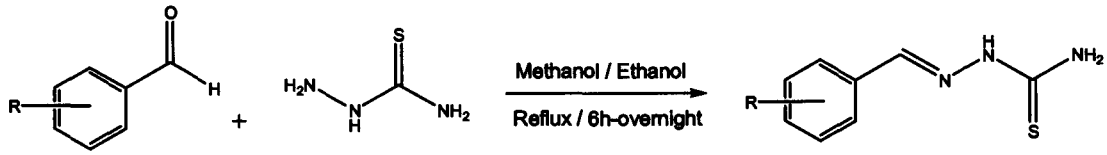
While the preparation of salicylaldehyde thiosemicarbazone metal complexes is well-documented, it is important to note that the only examples of palladium complexes in the literature are for salicylaldehyde derived ligands where the imine carbon is substituted with a H atom or methyl group[5,10,13] or one hydrogen of the terminal nitrogen in the thiourea moiety is substituted with a phenyl or methyl group.[12,15]

The preparation of palladium complexes of salicylaldimine thiosemicarbazone derivatives with different R-substituents on the phenol ring is, to the best of our knowledge, unknown. This chapter reports the synthesis and characterization of thiosemicarbazones from aldehydes with functionalized phenol rings and their corresponding palladium (II) complexes which will be fully discussed in this chapter.

2.2. Synthesis of Salicylaldimine Thiosemicarbazones

2.2.1. General Methods

The salicylaldimine thiosemicarbazones (**26** - **29**) were prepared by Schiff base condensation of a salicylaldehyde derivative with thiosemicarbazide (Scheme 2.1.).[16] The reaction mixture was refluxed in ethanol or methanol over times varying from 6 to 18 h. Thiosemicarbazide is only partially soluble in the reaction solvent at ambient temperature and heating to reflux facilitates complete dissolution of thiosemicarbazide.



26 R = 5-chloro-2-hydroxy
27: R = 3-tert. Butyl-2-hydroxy
28 R = 2-hydroxy-3-methoxy
29: R = 2-hydroxy

Scheme 2.1.

Upon cooling to room temperature the products precipitate out of solution, with the exception of **28** which begins to precipitate while the reaction is still at reflux temperature.

2.2.2. Physical Properties

Table 2.1. summarizes the physical properties of thiosemicarbazones **26** – **29**. The thiosemicarbazone ligands were isolated in moderate to good yields as air-stable white solids.

Table 2.1.: Physical Appearance, Yields and Melting Points of Ligands **26** - **29**

Ligand	Yield (%)	Physical Appearance	Melting Point (°C)
26	44	White solid	292-295
27	54	White solid	254-257
28	94	White solid	216-217
29	76	White solid	207-209

Thiosemicarbazones **26**, **28** and **29** are known compounds and their melting points agree with the literature.[17-19] All of the ligands exhibit high thermal stability, with melting points generally above 200 °C.

2.2.3. Spectroscopic and Analytical Characterization

The salicylaldimine thiosemicarbazone ligands (**26** – **29**) were characterized using Nuclear Magnetic Resonance spectroscopy (NMR) and infrared spectroscopy. For the known ligands, the proton NMR data corresponds with that of the literature.[14,18,19]

The ligand **27**, 3-^{t}butyl-2-hydroxy-benzaldehyde thiosemicarbazone, is a new compound and additional characterization was carried out in the form of elemental analysis and mass spectrometry.

Nuclear Magnetic Resonance (NMR) Spectroscopy

Thiosemicarbazones **26** - **29** are insoluble in most organic solvents and NMR experiments were run in DMSO-d_6. Table 2.2. summarizes the proton NMR data observed for all of the ligands. The imine proton for all of the ligands resonates as a singlet between 8.30 and 8.40 ppm and observation of this peak confirms the formation of a Schiff base.

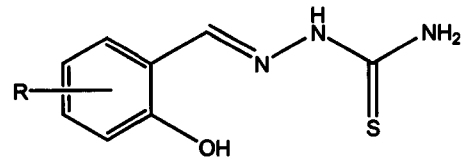


Table 2.2.: ^{1}H NMR Data for Thiosemicarbazones **26 – 29**

Ligand	R	H-C=N (ppm)	O-H (ppm)	N-H (ppm)	NH_2 (ppm)	Aromatic Protons (ppm)	R (ppm)
26	5-Cl	8.32	10.08	11.33	≈8.01[a]	8.01 - 6.87	–
27	3-^{t}Butyl	8.28	10.03	11.29	7.99	7.25 - 6.86	1.40 (*tert.*-butyl)
28	3-OMe	8.39	9.12	11.34	8.04; 7.82	7.50 - 6.75	3.79 (OMe)
29	H	8.35	9.83	11.31	8.04, 7.85 -7.88[b]	7.85 - 6.86	–

[a] Signal for these protons overlaps with one of the aromatic protons to occur as a singlet.
[b]The signal for one terminal amine proton overlaps with the signal of one of the aromatic protons to occur as a multiplet.

The hydroxyl and hydrazinic protons are observed as broad singlets further downfield relative to the imine proton. The hydrazinic proton exhibits similar resonances for all of the thiosemicarbazones while the hydroxyl proton of the ligand, 3-methoxy-2-hydroxy thiosemicarbazone (**28**), occurs slightly more upfield compared

to the ligands. The strong electron-donating nature of the methoxy substituent may account for this observation.

Two distinct singlets are observed for the amino, NH_2, protons for **28** at 8.04 and 7.82 ppm. This occurrence of two separate peaks for the amino protons is attributed to restricted rotation of the NH_2 group around the carbon nitrogen bond axis due to delocalisation of the lone pair of electrons of the amino nitrogen (Figure 2.2.).[20-22]

Figure 2.2. Depiction of the delocalization of the lone pair of electrons on -NH_2

The amino protons of **29** also show two peaks in the proton NMR spectrum, with one peak overlapping with the resonance of one aromatic proton giving rise to a multiplet between 7.85 and 7.88 ppm. The aromatic protons of the thiosemicarbazones (**26** – **29**) are observed in the expected region and the methyl protons of the tertiary butyl substituent of the aryl ring are observed as a singlet at 1.49 ppm.

The $^{13}C\{^1H\}$ NMR spectra for these ligands show similar shifts for the thione carbon. In fact, for ligands **27** – **29** the thione carbon is assigned to a signal seen at 177.8 ppm and for **26** it is observed at 178.0 ppm. Table 2.3. summarizes the carbon-13 data for the salicylaldimine thiosemicarbazones prepared. The imine carbon resonates between 137.6 and 147.0 ppm for all thiosemicarbazones. The influence of the high electron donation into the aromatic ring from the methoxy group of thiosemicarbazone **28** is again demonstrated by the upfield resonance of the hydroxyl substituted aromatic carbon, at 147.8, relative to the resonances observed for **26**, **27** and **29**.

Table 2.3.: $^{13}C\{^1H\}$ NMR Shifts for Thiosemicarbazones **26 – 29**

Ligand	C=N (ppm)	C=S (ppm)	C-OH (ppm)	$C_{(aromatic)}$ (ppm)	Other (ppm)
26	137.6	178.0	155.0	130.2, 125.4, 123.3, 122.2, 117.6	--
27	147.0	177.8	155.3	136.6, 129.3, 128.2, 119.1, 118.4	34.3 ($\underline{C}$-$(CH_3)_3$), 29.2 (C-$\underline{C}H_3$)
28	139.8	177.8	147.8	145.9, 120.6, 118.9, 118.2, 112.9	55.8 (O-$\underline{C}H_3$)
29	140.0	177.8	156.3	130.9, 126.8, 120.1, 119.1, 116.0	--

The chloro-substituted aromatic carbon of **26** is assigned to a peak seen at 123.3 ppm. For thiosemicarbazone **27**, the tertiary butyl substituted aromatic carbon resonates at 136.6 ppm. The quaternary carbon of the tertiary butyl group is observed at 34.3 ppm and the methyl carbons occur at 29.2 ppm.

Infrared (IR) Spectroscopy

Infrared spectra for the salicylaldimine thiosemicarbazones (**26 – 29**) were run as KBr pellets and selected IR absorptions are shown in Table 2.4. The presence of an absorption band between 1598 and 1615 cm^{-1} in the spectrum of each compound corresponds to the C=N stretching frequency and confirms formation of the thiosemicarbazone product.

Table 2.4.: Selected IR Absorptions for Thiosemicarbazones **26 – 29**

Ligand	N-H (cm^{-1})	O-H (cm^{-1})	C=N (cm^{-1})	C=S (cm^{-1})
26	3236[b,c], 3165[a], 3045[d]	3405[c]	1612[c]	1099[c]
27	3248[a], 3164[a], 3038[c]	3420[a]	1614[a]	1148[c]
28	3343[a], 3167[a], 3033[c]	3461[a]	1598[a]	1060[c]
29	3318[a], 3173[a], 3029[b,c]	3443[c]	1615[a]	1111[c]

[a] strong intensity; [b] broad; [c] medium intensity; [d] weak intensity

The absorption band corresponding to the O-H vibration is observed with strong intensity for compounds **27** and **28**, at 3420 and 3461 cm^{-1} respectively; with medium intensity at 3407 cm^{-1} for **26** and 3443 cm^{-1} for **29**.[23] In the infrared spectrum of each compound, three absorption bands are observed between 3320-3000 cm^{-1} with

varying intensity. The two bands occurring at the higher frequency, *ca.* 3150 – 3320 cm^{-1}, in each ligand are assigned to the N-H bond vibrations of the terminal amine group. The lower frequency band is therefore due to the hydrazinic N-H bond vibration.[5,24]

Assignment of the thiocarbonyl (C=S) vibration is sometimes difficult since it has a less polar bond than the analogous C=O group and therefore occurs at a lower frequency where it is susceptible to coupling effects.[25] In addition to this, compounds where the C=S group is attached to a nitrogen atom not only show an absorption band in the characteristic thione region (1250–1020 cm^{-1}) but several bands may be observed between 1563-700 cm^{-1} due to vibrations involving interaction between the C-N and C=S bonds.[25]

The stretching frequency of the thione functionality for thiosemicarbazones **26** - **29** was thus assigned to the strongest band observed in this region of the spectra, between 1050 and 1150 cm^{-1}, with the highest frequency band observed for 3-*tert.*-butyl-2-hydroxy benzaldehyde thiosemicarbazone (**27**).[22]

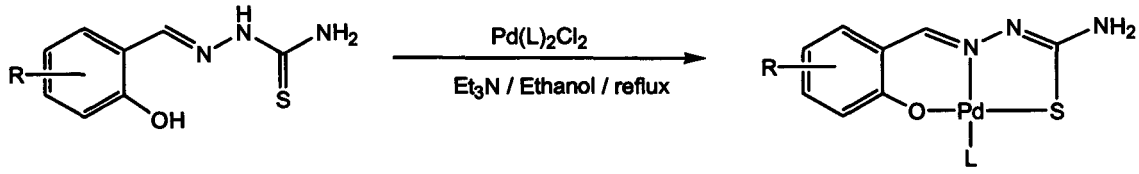
Mass Spectrometry and Elemental Analysis

Since thiosemicarbazones **26**, **28** and **29** are known and their proton NMR data and melting points agree with their literature values, elemental analysis was only determined for thiosemicarbazone **27**. The ligand was analysed for C, H, N and S and found to be within acceptable limits to that of the the calculated values. ESI-mass spectrometry for 3-^{t}butyl-2-hydroxy-benzaldehyde thiosemicarbazone (**27**) shows a base peak at *m/z* 252.1 corresponding to the ligand in its protonated form, $[M + H]^{+}$.

2.3. Synthesis of Salicylaldiminato Thiosemicarbazone Pd(II) Complexes

2.3.1. General Methods

The salicylaldimine thiosemicarbazone ligands (**26 – 29**) were reacted with either, dichlorobis(triphenylphosphine)palladium(II) or dichlorobis(4-picoline)palladium(II) (Scheme 2.2.)[10] to give rise to eight salicylaldiminato thiosemicarbazone palladium complexes (**26a – 29b**), of which six are new complexes (**26a – 28a** and **26b - 28b**).



26: R = 5-chloro
27: R = 3-tert. Butyl
28: R = 3-methoxy
29: R = H

26a - 29a: L = PPh_3
26b - 29b: L = 4-picoline

Scheme 2.2.

A suspension of the appropriate thiosemicarbazone ligand in dry ethanol is heated to 60 °C to facilitate dissolution of the ligand. Triethylamine and $[Pd(L)_2Cl_2]$ (L = PPh_3 or 4-picoline) were then added. The reaction was stirred at reflux over times varying from 4 to 6 hours.

2.3.2. Physical Properties

The palladium complexes **26a – 29b** were isolated as air- and moisture- stable orange or yellow solids in low to moderate yields. Table 2.5. summarizes the yields and physical properties. All of the complexes show good thermal stability, melting at temperatures higher than 180 °C. The complexes where triphenylphosphine is the co-ligand (**26a -29a**) show melting points between 220 and 250 °C. No melting or decomposition was observed for **27b** even at temperatures greater than 310 °C.

Table 2.5.: Summary of Physical Properties of Complexes **26a – 29b**

Complex	Yield (%)	Physical Appearance	Melting Point (°C)
26a	63	Crystalline yellow solid	221-223
27a	29	Crystalline orange solid	234-236
28a	80	Dark orange crystalline solid	241-243
29a	36	Crystalline orange solid	228-231
26b	30	Yellow solid	decomposition without melting 238-240
27b	46	Yellow solid	No melting or decomposition up to 310
28b	38	Crystalline orange solid	225-227
29b	34	Orange Solid	decomposition without melting 185-187

2.3.4. Spectroscopic and Analytical Characterization

All of the complexes were characterized using NMR and infrared spectroscopy. The complexes **26a** – **28a** and **26b** – **28b** are new compounds and were further characterized using mass spectrometry and elemental analysis. Complexes **29a** and **29b** are known and their ^{1}H NMR spectra agree with the literature.[10,11]

Nuclear Magnetic Resonance (NMR) Spectroscopy

The salicylaldiminato thiosemicarbazone Pd(II) complexes are soluble in most organic solvents and NMR experiments were recorded in chloroform-d_1. Table 2.6. summarizes the proton and phosphorus NMR data for the complexes **26a** – **29a**; the proton NMR data for complexes **26b- 29b** are shown in Table 2.7.

For complexes **26a** – **29a**, the imine proton resonates as a doublet between 8.13 and 8.27 ppm. The splitting of this signal is evidence of coordination of the imine nitrogen to palladium resulting in long range coupling of the imine proton to the phosphorus nucleus of triphenylphosphine.[10,26] A ^4J(PH) coupling constant of approximately 14.00 Hz further supports phosphorus-proton coupling effects and is consistent with coordination of triphenylphosphine *trans* to the imine nitrogen in complexes **26a** – **29a**[26,27] which would be expected if the thiosemicarbazone ligand bonds to palladium as a tridentate [O,N,S] donor.

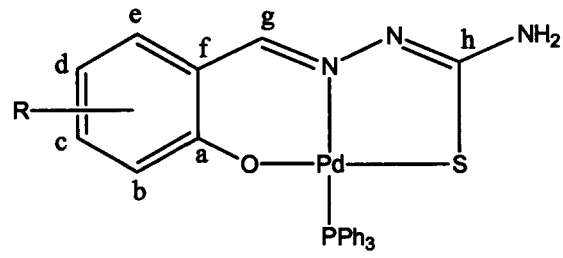


Table 2.6.: ^{1}H and $^{31}P\{^{1}H\}$ NMR data for complexes **26a - 29a**

		^{31}P	^{1}H				
Complex	**R**		**H-C=N (ppm)**	**NH_2 (ppm)**	**Aromatic Protons (ppm)**	**R (ppm)**	**PPh_3 (ppm)**
26a	5-Cl	25.23	8.13 (d, $^{4}J_{PH}$ = 13.62 Hz)	4.74 (s)	7.23 (d, $^{4}J(H_eH_c)$ = 2.75, 1H, H_e), 7.11 (d, $^{3}J(H_cH_b)$ = 9.02 Hz, 1H, H_c), 6.59 (d, $^{3}J(H_bH_c)$ = 9.02 Hz, 1H, H_b)	–	7.26-7.55, 7.66-7.75
27a	3-^{t}Butyl	24.16	8.26 (d, $^{4}J_{PH}$ = 14.14 Hz)	4.59 (s)	7.20-7.37 (m, 2H, H_c, H_e), 6.57 (t, $^{3}J(H_cH_dH_e)$ = 8.82 Hz, 1H, H_d)	0.74 (s)	7.38-7.48, 7.70-7.77
28a	3-OMe	19.67	8.24 (d, $^{4}J_{PH}$ = 13.87 Hz)	4.67 (s)	6.96 (d, $^{3}J(H_eH_d)$ = 8.06 Hz, 1H, H_e), 6.85 (d, $^{3}J(H_cH_d)$ = 7.57 Hz, 1H, H_c), 6.56 (t, $^{3}J(H_cH_dH_e)$ = 7.79 Hz, 1H, H_d)	3.57 (s)	7.26-7.49, 7.49-7.81
29a	H	24.97	8.27 (d, $^{4}J_{PH}$ = 13.50 Hz)	4.69 (s)	7.28-7.43 (m, 3H, H_b, H_c, H_e)	–	7.70-7.75, 7.50-7.53

s = singlet, d = doublet, t = triplet, m = multiplet

Disappearance of the signals corresponding to the hydrazinic proton and the phenolic proton in the free ligand confirms the nature of coordination of the thiosemicarbazone ligand to palladium; as a dianionic (via phenolic oxygen and thiolate sulfur) tridentate donor. The terminal amine, NH_2, protons are observed as singlets upfield relative to the aromatic protons between 4.50 and 4.75 ppm for **26a – 29a**. The aromatic protons of triphenylphosphine and the thiosemicarbazone ligand are seen in the expected region of the spectrum.[10,12]

In the phosphorus NMR spectra, complexes **26a**, **27a** and **29a** exhibit a singlet between 24.00 and 26.00 ppm.[26] For complex **28a**, the phosphorus nucleus

resonates further upfield at 19.67 ppm. This shielding is attributed to the electron-donating nature of the methoxy substituent which would result in greater electron density around palladium and hence greater back bonding between palladium and phosphorus.

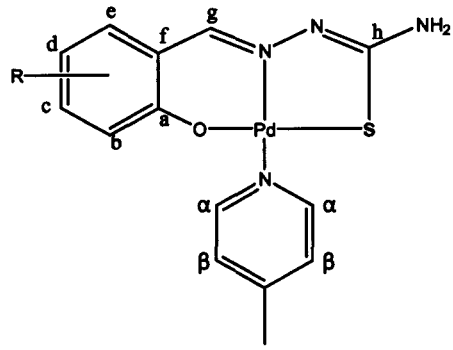


Table 2.7.: ^{1}H NMR Data for complexes **26b - 29b**

Complex	R	H-C=N (ppm)	NH_2 (ppm)	Aromatic Protons (ppm)	R (ppm)	4-Picoline (ppm)
26b	5-Cl	7.83 (s)	4.78 (s)	7.16-7.44[a], 6.94 - 6.96 (m, 2H, H_b, H_c)	--	2.44 (CH_3), 8.59 (d, 3J(H_αH) = 6.60 Hz, 2H, H_α), 7.16-7.44(H)[a]
27b	3-^{t}Butyl	7.95 (s)	4.73 (s)	7.32-7.36 (m, 1H, H_e), 7.18-7.25[a], 6.79-6.42 (m, 1H, H_c)	1.33 (s)	2.47(CH_3), 8.69 (d, ^3J(H_αH) = 6.50 Hz, 2H, H_α), 7.18-7.25 (H_β)[a]
28b	3-OMe	7.92 (s)	4.74 (s)	7.14-7.31 (m, 1H, H_e), 6.95 -7.02[a], 6.52-6.68 (m, 1H, H_c)	3.85 (s)	2.43(CH_3), 8.70 (d, ^3J(H_αH) = 6.60 Hz, 2H, H_α), 6.95 - 7.02(H)[a]
29b	H	7.98 (s)	4.85 (s)	7.32-7.39 (m, 2H, H_c, H_e), 7.09 (d, ^3J(H_bH_c) = 8.95, 1H, H_b), 6.73 (t, ^3J($H_cH_dH_e$) = 7.38, 1H, H_d)	--	2.49(CH_3), 8.68 (d, ^3J(H_αH) = 6.57 Hz, 2H, H_α), 7.29 (d, 3J(H H_α) = 5.96 Hz, 2H, H)

[a] One aromatic proton of the thiosemicarbazone ligand overlaps with the signal for the aromatic protons beta to nitrogen of the picoline ring; s = singlet, d = doublet, t = triplet, m = multiplet

In the proton NMR spectra of complexes **26b** – **29b** (Table 2.7.), the imine proton occurs at a lower shift compared to complexes **26a** – **29a**. This shielding of the proton is expected as the 4-picoline co-ligand is a better sigma-donor towards

palladium than triphenylphosphine. The shifts observed for the terminal amine protons in **26b** – **29b** are similar to that of **26a** – **29a** suggesting that there is no significant effect on this group from the co-ligand. The protons of the tertiary butyl (**27b**) and methoxy (**28b**) substituents appear to be slightly more deshielded compared to its triphenylphosphine analogues (**27a** and **28a** respectively).

As with complexes **26a** – **29a**, the resonances attributed to the phenolic and hydrazinic protons in the free ligand are not observed in the spectra of complexes **26b** – **29b** supporting coordination of the thiosemicarbazones in the same tridentate [O,N,S] manner. The protons of the methyl substituent of the picolyl ring resonate as a singlet at ca. 2.40 ppm for **26b** – **29b**. The signal of one aromatic proton of the thiosemicarbazone ligand overlaps with the signal of the protons beta to nitrogen in the picolyl ring of the co-ligand to occur as a multiplet in complexes **26b** – **29b**. The protons, H_α of the picolyl ring are seen as a doublet at 8.59 ppm for **26b** due to coupling to the adjacent proton, H . This pattern is also observed in the spectra of the complexes **27b** – **29b**.

Table 2.8. lists the resonances observed in the $^{13}C\{^1H\}$ NMR spectra for all complexes, **26a** – **29b**. The imine carbon of the thiosemicarbazone ligand resonates at approximately 170.0 ppm in all of the complexes. This shift is characteristic of coordination of the imine nitrogen to palladium leading to a downfield shift of this carbon relative to the resonance observed for the free ligand.[15,24,28] The thiolate carbon generally occurs between 161.0 and 162.0 ppm for all of the complexes except **28a** and **28b**, where it resonates at 150.9 and 153.8 ppm respectively. It seems that in these complexes there is greater shielding of the C-S carbon due to the methoxy substituent of the thiosemicarbazone ligand. Overall, the upfield shift of the C-S carbon in all complexes compared to the thione carbon of the corresponding free ligand is evidence of coordination of sulfur to palladium in the thiolato form since shielding of the thiolate carbon is characteristic of this type of bonding.[24,29-31]

Table 2.8.: $^{13}C\{^1H\}$ data for complexes **26a - 29b**

Complex	C=N (ppm)	C-S (ppm)	C-O (ppm)	Aromatic Carbons (ppm)	Other (ppm)
26a	171.5	161.2	149.6	128.3-134.7 (PPh_3, C_c, C_d and C_e), 122.2 (C_f), 118.7 (C_b)	–
27a	170.0	162.2	152.3	140.3 (C_b), 128.0-135.1 (PPh_3, C_e, C_c), 118.0 (C_d), 114.1 (C_f)	34.8 ($\underline{C}$-(CH_3)$_3$), 29.4 ((C-($\underline{C}H_3$)$_3$)
28a	170.4	≈150.9[a]	Unassigned[b]	128.3-134.8 (PPh_3, C_a), 126.5 (C_e), 117.7 (C_d), 115.5 (C_f), 113.9 (C_c)	56.6 (O-$\underline{C}H_3$)
29a	171.0	162.7	150.7	128.3-134.7 (PPh_3, C_e, C_c), 120.7 (C_d), 117.6 (C_f), 114.8 (C_b)	–
26b	172.7	161.1	152.3	151.8 (C_a), 132.7 (C_d), 132.3 (C_e), 127.5 (C), 121.9 (C_c), 120.5 (C_f), 117.9 (C_b)	21.3 ($\underline{C}H_3$, Picoline)
27b	169.8	161.8	152.0	151.7 (C_a), 138.9 (C_b), 132.6 (C_c), 130.3 (C_e), 125.9 (C), 118.7 (C_d), 114.3 (C_f)	35.2 ($\underline{C}$-(CH_3)$_3$), 29.4 (C-($\underline{C}H_3$)$_3$, 21.2 ($\underline{C}H_3$, Picoline)
28b	170.5	153.8	149.0	151.8 (C_a), 152.2 (C_b), 127.4 (C), 126.2 (C_e), 125.3 (C_d), 119.1 (C_f), 114.5 (C_c)	56.7 (O-$\underline{C}H_3$), 21.2 ($\underline{C}H_3$, Picoline)
29b	170.0	162.6	151.6	151.0 (C_a), 133.9 (C_c), 133.6 (C_e), 126.3 (C), 119.9 (C_d), 118.4 (C_f), 115.1 (C_b)	21.2 ($\underline{C}H_3$, Picoline)

[a] Signal for the thiolate carbon overlaps with that of the methoxy substituted aromatic carbon to occur as a singlet.
[b] unable to assign as the signal overlaps with the signals for triphenylphosphine

The resonances for the aromatic carbons for all complexes are observed in the characteristic region of the spectrum. The ^{t}butyl group in **27a** and **27b** shows similar resonances in both complexes and this is also observed for the carbon of the methoxy substituent in complexes **28a** and **28b**.

Infrared (IR) Spectroscopy

Infrared spectra for all of the palladium complexes were recorded as KBr pellets. Selected IR absorptions are listed in Table 2.9. for **26a – 29b**.

Table 2.9.: IR absorptions for the imine and amine vibrations for **26a – 29b**

Complex	C=N (cm^{-1})	N-H (cm^{-1})
26a	1605, 1586	3493, 3385
27a	1634, 1610	3464, 3391
28a	1642, 1592	3442, 3308
29a	1638, 1599	3300, 3132
26b	1622, 1596	3403, 3273
27b	1621, 1609, 1591	3421, 3310
28b	1621, 1603	3428, 3351
29b	1625, 1600, 1588	3434, 3278

In the infrared spectra for complexes **26a – 29a** two bands are observed in the imine region, this is consistent with the formation of a new imine bond within the thiosemicarbazone ligand upon coordination of sulfur in the thiolate form. The lower frequency band, observed between 1610 and 1585 cm^{-1}, is assigned to the imine bond coordinated to palladium.[13,14,29] When the nitrogen of the imine coordinates to the metal, a loss of double bond character occurs due stronger electron donation towards the metal, thus this bond will vibrate at a lower frequency.

For complexes **26b–29b**, high frequency bands observed between 1625 and 1620 cm^{-1} are assigned to the new imine bond formed. The vibration of the coordinated imine for these complexes is harder to assign as the signal for this vibration may overlap with the imine vibration of the 4-picolyl ring. In complexes **27b** and **29b**, there are three distinct bands in the imine region and the coordinated imine vibration is assigned to the bands observed at 1609 cm^{-1} for **27b** and at 1600 cm^{-1} for **29b**. The C=N stretch of the 4-picolyl ring is allocated to the lowest frequency band observed. In the spectra of complexes **26b** and **28b**, the absorption bands observed at 1596 cm^{-1} for **26b** and 1603 cm^{-1} for **28b** are slightly broad and this may indicate the overlapping of two C=N vibrations resulting in only one absorption band being observed.

In all of the complexes two absorption bands are observed for the N-H vibrations of the terminal amine between 3500 and 3250 cm^{-1}. A third absorption band for the hydrazinic N-H bond is not observed and this is consistent with the loss of the hydrazinic proton and formation of a new imine bond in the thiosemicarbazone ligand.

Mass Spectrometry and Elemental Analysis

The formation of the salicylaldiminato thiosemicarbazone Pd(II) complexes was further confirmed using ESI-mass spectrometry run in the positive mode (Table 2.10.) and elemental analysis. As **29a** and **29b** are known and NMR data corresponded to the literature,[10,11] these complexes were not submitted for further characterization.

Table 2.10.: Mass Spectral Results for Complexes **26a – 28b**

Complex	Calculated Molar Mass (g/mol)	Molecular Fragment	Assignment
26a	596.37	597	$[M + H]^+$
27a	618.04	617	$[M - H]^+$
28a	591.96	591	$[M - H]^+$
26b	427.22	429	$[M+2H]^{2+}$
27b	448.88	449	$[M + H]^+$
28b	422.80	423	$[M + H]^+$

All of the complexes showed a base peak corresponding to the calculated molar mass with either loss or gain of a proton. Elemental analysis results are in within acceptable limits compared to the calculated values for all complexes.

2.4. X-ray Crystallography

Single crystals suitable for X-ray diffraction of the Pd(II) complexes **26a – 28a** were grown by slow-evaporation of a 1:1 DCM-hexane or $CDCl_3$-hexane solvent system. Table 2.11. summarizes the crystal data for each molecular structure and Table 2.12. lists selected bond lengths and angles of the molecular structures.

Table 2.11.: Crystallographic Data of Complexes **26a – 27a**

	26a	**27a**	**28a**
Empirical formula	$C_{27}H_{23}Cl_3N_3OPPdS$	$C_{32}H_{30}Cl_6D_2N_3OPPdS$	$C_{27}H_{24}N_3O_2PPdS$
Empirical Weight	681.26	858.75	591.92
Crystal size	0.20 x 0.14 x 0.08 mm	0.18 x 0.14 x 0.14 mm	0.12 x 0.11 x 0.09 mm
Crystal system, space group	Monoclinic, $P2_1/c$	Monoclinic, $P2_1/n$	Monoclinic, $C2/c$
a	14.7145(4) Å	10.1900(2) Å	33.3399(7) Å
b	8.0414(1) Å	20.5408(4) Å	10.3708(2) Å
c	24.3591(6) Å	17.9344(3) Å	14.9613(2) Å
alpha	90°	90°	90°
beta	101.151°	102.543°	102.0910°
gamma	90°	90°	90°
Volume	2827.88(11) $Å^3$	3664.27(12)$Å^3$	5058.28(16) $Å^3$
Z	4	4	8
Calculated density	1.600 Mg/m^3	1.557 Mg/m^3	1.555 Mg/m^3
F(000)	1368	1728	2400
R indices (all data)	R1 = 0.0528, wR2 = 0.0883	R1 = 0.0476, wR2 = 0.0887	R1 = 0.0362, wR2 = 0.0628

Complex **26a** (Fig. 2.2.) crystallized with a monoclinic system and space group $P2_1/c$. There is one molecule of dichloromethane solvent included in the structure per complex molecule in **26a** and two chloroform molecules included in the crystal per complex molecule for **27a**. Figures 2.3. and 2.4. show the molecular structures of complexes **27a** and **28a** respectively. In all of the complexes, the thiosemicarbazone ligand coordinates to palladium in the expected tridentate fashion, via the phenolate oxygen, imine nitrogen and thiolate sulfur forming five- and six-membered chelate rings with the metal. The fourth coordination site is occupied by the triphenylphosphine co-ligand *trans* to the imine nitrogen.

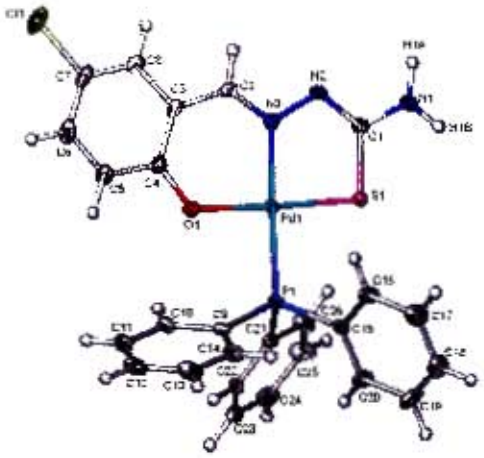


Figure 2.2.: Molecular structure of complex **26a** with ellipsoidal model of probability level = 40%. The solvent molecule CH_2Cl_2 is excluded.

Figure 2.3.: Molecular structure of complex **27a** with ellipsoidal model of probability level = 40%. All H are omitted for clarity. Two solvent molecules $CDCl_3$ are excluded.

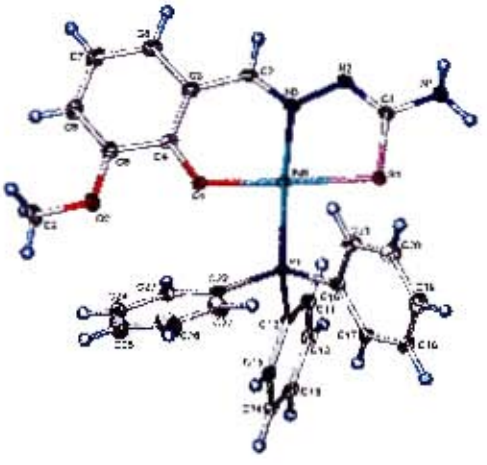


Figure 2.4.: Molecular structure of complex **28a** with ellipsoidal model of probability level = 40%.

The bite angles formed between the metal and the coordinated ligands show that in each complex there is a slightly distorted square-planar arrangement around the metal. All of the bite angles around the metal in each complex are consistent with those observed for similar complexes.[10-12] The bite angle that shows the least deviation from 90° is the P(1)-Pd(1)-O(1); 89.41(7) for **26a**, 90.07(6) for **27a** and 90.43(5) for **28a**. The *trans* angles O(1)-Pd(1)-S(1) and N(3)-Pd(1)-P(1) are close to linearity in all of the molecular structures with **26a** showing the least deviation from 180°.

Table 2.12.: Selected Bond Lengths and Angles for Complexes **26a – 28a**

	26a	**27a**	**28a**
Pd(1)-S(1)	2.2432(8) Å	2.2426(8) Å	2.2478(6) Å
Pd(1)-P(1)	2.2833(8) Å	2.2782(8) Å	2.2779(6) Å
Pd(1)-N(3)	2.028(3) Å	2.012(2) Å	2.0204(19) Å
Pd(1)-O(1)	2.014(2) Å	2.033(2) Å	2.0164(16) Å
C(1)-S(1)	1.754(3) Å	1.754(3) Å	1.751(3) Å
C(1)-N(2)	1.303(4) Å	1.304(4) Å	1.291(3) Å
C(2)-N(3)	1.294(4) Å	1.293(4) Å	1.294(3) Å
N(3)-Pd(1)-S(1)	84.16(8)°	84.52(7)°	84.09(6)°
N(3)-Pd(1)-P(1)	177.29(8)	175.78(7)	175.91(6)
N(3)-Pd(1)-O(1)	93.03(10)°	92.15(9)°	93.15(7)°
P(1)-Pd(1)-S(1)	93.41(3)°	93.56(3)°	92.27(2)°
O(1)-Pd(1)-S(1)	177.14(7)°	174.07(6)	176.51(5)
P(1)-Pd(1)-O(1)	89.41(7)°	90.07(6)°	90.43(5)°

Inspection of the bond angles formed between the metal and the coordinated atoms show that they are consistent with analogous complexes.[10,11] The Pd(1)-N(3) bond distance observed in **26a** and **28a** are slightly longer than that of **27a**. This suggests there may be greater *trans* influence exerted by the triphenylphosphine ligand in these two complexes.[12]

The C(1)-S(1) bond has a length of approximately 1.75 Å for all three complexes, closer to the expected bond length of a typical C-S single bond (1.82 Å) than that of a C=S double bond (1.56 Å),[32] confirming that sulfur coordinates to palladium in the thiolate form.[33] Further evidence of this is obtained from the bond distances observed for the C(1)-N(2) bond. The approximate bond length of 1.30 Å in all of the molecular structures is similar to that of the C(2)-N(3) imine bond, indicating increased double bond character and formation of a new double bond between

carbon and nitrogen in the thiosemicarbazone ligand upon coordination to palladium.[10,15,34]

2.6. Conclusion

Salicylaldimine thiosemicarbazones (**26** – **29**) were prepared by Schiff-base condensation reactions of thiosemicarbazide and the appropriate salicylaldehyde derivative and isolated as air stable solids. These ligands were reacted with dichlorobis(triphenylphosphine)palladium(II) and dichlorobis(4-picoline)palladium(II) to yield eight salicylaldiminato thiosemicarbazone Pd(II) complexes (**26a-29b**), six of which are new compounds.

Spectroscopic characterization of these complexes support coordination of thiosemicarbazone as a dianionic tridentate [O,N,S] donor to palladium. This was further supported by the molecular structures of **26a** – **28a** which were ascertained using single crystal X-ray diffraction methods.

2.7. References

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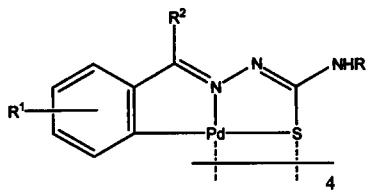
Chapter 3: Towards the Synthesis of Tridentate [C,N,S] Monothiosemicarbazone Palladium(II) Complexes

3.1. Introduction

Aryl-derived monothiosemicarbazones are capable of coordinating to palladium as [C,N,S] tridentate donors via sulfur, imine nitrogen and the C-H activated aromatic *ortho*-carbon.[1] These types of Pd(II) compounds are also known as cyclopalladated or *ortho*-palladated complexes. Tetranuclear thiosemicarbazone palladium(II) complexes have been reported (**14**, Figure 3.1.).[2-6] The thiosemicarbazone ligand forms two fused rings with the metal center and Pd-$S_{bridging}$ coordination bonds give rise to a Pd_4S_4 core.



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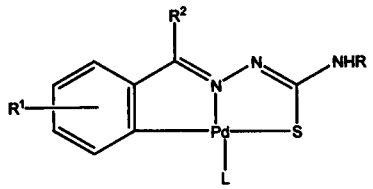
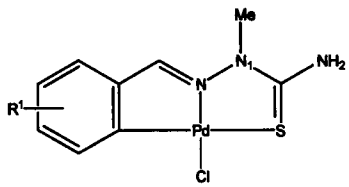


15: L = tertiary phosphine



18

Figure 3.1.: Examples of the variety of palladium complexes prepared from aryl mono-thiosemicarbazones

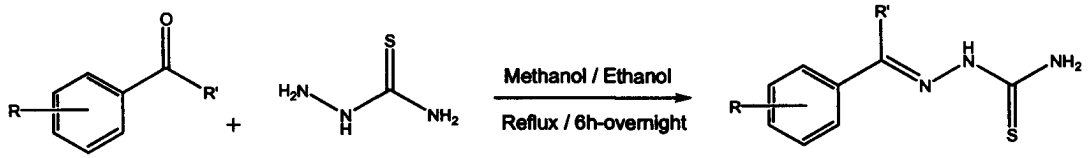
When the tetranuclear complex is reacted with a nucleophile such as a tertiary phosphine the palladium-sulfur bridging bond is cleaved, yielding a mononuclear *ortho*-palladated complex (**15**). Substitution of the hydrazinic nitrogen (N_1) with a methyl group hinders formation of the thiolate and the ligand acts as a uninegative [C,N,S] donor yielding a mononuclear complex (**18**)[2] since the thiolate sulfur is fundamental for the formation of the tetranuclear structure.

In this chapter, the synthesis and characterization of five aryl mono-thiosemicarbazones and their corresponding palladium(II) complexes will be presented. All of the ligands have previously been synthesized.[7,8] In addition they have shown activity as inhibitors of the *Trypanosoma cruzi* cysteine protease cruzain.[7] This established bioactivity has led to an interest in the preparation and application of their palladium(II) complexes as possible biological agents. Cyclopalladation of these thiosemicarbazones by C-H activation of the *ortho*-carbon was undertaken and proved successful for two of the ligands. The remaining three thiosemicarbazones exhibited a different coordination mode toward palladium and the results are fully discussed.

3.2. Preparation of Aryl Monothiosemicarbazones

3.2.1. General Methods

Ligands **30** – **34** (Scheme 3.1.) were prepared using the same method of preparation as that of thiosemicarbazone ligands **26** – **29**, outlined in Chapter 2 (Section 2.2.1.). The appropriate aryl aldehyde or ketone was condensed with thiosemicarbazide in methanol or ethanol. Acetic acid was added for those condensation reactions where a ketonic starting material was used, since the carbonyl functional group is generally less reactive than its aldehyde analogue.



30: R = 3,4-dichloro; R' = CH_2CH_3
31: R = 3,4-dichloro; R' = CH_3
32: R = 3,4-dichloro; R' = H
33: R = 3,5-bis(trifluoromethyl); R' = CH_3
34: R = 3,5-bis(trifluoromethyl); R' = H

Scheme 3.1.

3.2.2. Physical Properties

Ligands **30** – **34** were isolated as white solids in moderate to low yields. Table 3.1. lists their physical appearance and melting points which correspond to the literature.[7,8]

Table 3.1.: Physical appearance, yields and melting points for **30** – **34**

Ligand	R	R'	Yield (%)	Physical Appearance	Melting Point (°C)
30	3,4-dichloro	CH_2CH_3	21	White solid	188-190
31	3,4-dichloro	CH_3	35	White solid	191-192
32	3,4-dichloro	H	13	White solid	195-198
33	3,5-bis(trifluoromethyl)	CH_3	41	White solid	decomp. 240-241
34	3,5-bis(trifluoromethyl)	H	27	White solid	225-227

All of the ligands exhibit high thermal stability with compounds **30** – **32** and **34** melting at temperatures greater than 180 °C; ligand **33** decomposes between 240 - 241 °C. The ligands show poor solubility in most common organic solvents. They were found to be soluble in dimethyl sulfoxide (DMSO) as well as water.

3.2.3. Spectroscopic Characterization

Since ligands **30** – **34** are known compounds, they were characterized using only Nuclear Magnetic Resonance (NMR) and infrared (IR) spectroscopies. The data obtained corresponds to the literature values and confirm the integrity of these molecules.[7,8]

Nuclear Magnetic Resonance (NMR) Spectroscopy

^1H and $^{13}C\{^1H\}$ NMR as well as $^{19}F\{^1H\}$ NMR spectra were recorded for ligands **33** and **34**. Table 3.2. summarizes proton (**30** – **34**) and fluorine (**33** and **34**) NMR data. For all of the ligands, the hydrazinic proton is observed as a singlet at downfield resonances between 10 – 12 ppm and the terminal amine protons occur between 8.10 and 8.50 ppm.

The imine proton of ligand **32** resonates at a similar frequency as the terminal amine protons and the signals for these three protons overlap to appear as a singlet. Similar to ligands **28** and **29**, discussed in Chapter 2 (Section 2.2.3), ligands **30** and **34** show two distinct singlets for the $-NH_2$ protons. This observation is also attributed to restricted rotation of the $-NH_2$ group around the carbon nitrogen bond axis due to the delocalisation of the lone pair of electrons of the amino nitrogen.[9-11]

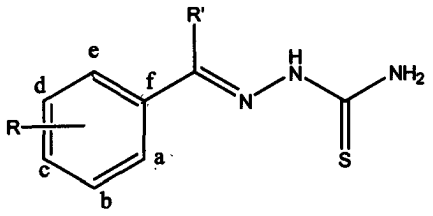


Table 3.2.: ^1H and $^{19}F\{^1H\}$ NMR Data for Ligands **30 – 34**

				^1H				^{19}F
Ligand	**R**	**R'**	**H-C=N (ppm)**	**N-H (ppm)**	**NH_2 (ppm)**	**Aromatic protons**	**Other (ppm)**	**CF_3 (ppm)**
30	**3,4-dichloro**	**Et**	-	10.36 (s)	8.31 (s), 8.13 (s)	8.23 (s, H_a), 7.86 (dd, $^4J(H_eH_a)$ = 2.80, $^3J(H_eH_d)$ = 8.55 Hz, H_e), 7.61 (d, $^3J(H_dH_e)$ = 8.54 Hz, H_d)	2.86 (q, CH_2), 0.98 (t, CH_3)	-
31	**3,4-dichloro**	**Me**	-	10.20 (s)	8.13 (s)	8.26 (s, H_a), 7.90 (d, $^3J(H_dH_e)$ = 8.52 Hz, H_d), 7.66 (d, $^3J(H_eH_d)$ = 8.52 Hz, H_e)	2.33 (s, CH_3)	-
32	**3,4-dichloro**	**H**	≈8.21 (s)	11.51 (s)	≈8.21 (s)	7.98 (s, H_a), 7.71 (d, $^3J(H_dH_e)$ = 8.37 Hz, H_d), 7.63 (d, $^3J(H_eH_d)$ = 8.33 Hz, H_e)	-	-
33	**3,5-bis(trifluoro-methyl)**	**Me**	-	10.33 (s)	8.33 (s)	8.52 (s, 2H, H_a, H_e), 8.05 (s, 1H, H_c)	2.34 (s)	-61.59 (s)
34	**3,5-bis(trifluoro-methyl)**	**H**	8.16 (s)	11.68 (s)	8.43 (s), 8.34 (s)	8.52 (s, 2H, H_a, H_e), 8.03 (s, 1H, H_c)	-	-61.74 (s)

s = singlet, d = doublet, dd = doublet of doublets, t = triplet and q = quartet

The imine proton for ligand **34** resonates at 8.16 ppm as a singlet. For ligands **30 – 32**, where the R substituents on the aryl ring are chlorido groups in the 3 and 4 positions, the three aromatic protons show distinct signals between 7.60 and 8.30 ppm. The furthest upfield resonance (*ca.* 7.60 ppm) of the aromatic protons is

assigned to the proton in the 5-position (H_e) and the proton (H_a) bonded to the C-2 carbon of the ring is assigned to the most downfield shift.

For thiosemicarbazones **33** and **34**, where trifluoromethyl groups are bonded in the 3 and 5 positions of the aryl ring, only two singlets are observed for the aromatic protons since two of the protons are chemically equivalent. For both ligands, the protons H_a and H_e occur at 8.52 ppm as a singlet. The $^{19}F\{^1H\}$ NMR spectrum of ligands **33** and **34** show a singlet at -61.59 and -61.74 ppm respectively, evidence that only one product is isolated.

Table 3.3. summarises the $^{13}C\{^1H\}$ NMR data for thiosemicarbazones **30** – **34**. For all of the ligands, the thione carbon is observed at a similar shift (*ca.* 179.0 ppm) and the imine carbon exhibits a peak between 139.0 and 149.0 ppm.

Table 3.3.: $^{13}C\{^1H\}$ NMR Shifts Observed for Ligands **30** – **34**

Ligand	C=N (ppm)	C=S (ppm)	$C_{(aromatic)}$ (ppm)	Other (ppm)
30	149.3	179.2	136.9 (C_f), 131.5 (C_c), 131.4 (C_b), 130.2 (C_a), 128.2 (C_d), 126.7 (C_e)	18.9 (CH_2), 10.5 (CH_3)
31	145.2	179.1	138.2 (C_f), 131.5 (C_c), 131.2 (C_b), 130.0 (C_d), 128.0 (C_a), 126.5 (C_e)	13.6 (CH_3)
32	139.4	178.3	134.9 (C_c), 131.7 (C_b), 131.6 (C_f), 130.5 (C_d), 128.0 (C_a), 127.4 (C_e)	-
33	145.9	180.1	141.2 (C_f), 131.3 (C_b, C_d), 127.9 (C_a, C_e), 125.4 (C_c)	122.7 (CF_3), 14.9 (CH_3)
34	139.7	179.4	138.0 (C_f), 132.0 (C_b, C_d), 128.5 (C_a, C_e), 125.4 (C_c)	123. 0 (CF_3)

The aromatic carbons of **30** – **32** show six distinct peaks between 130.0 and 126.0 ppm. For ligands **33** and **34** the trifluoromethyl substituted carbons are observed at

131.3 and 132.0 ppm respectively. The *ortho* carbons occur at *ca.*128 ppm and the fluorine substituted carbons show a peak at approximately 123 ppm. The methyl carbon of the imine subtituents for ligands **31** and **33** are observed at 13.6 and 14.9 ppm respectively, while the $-CH_2-$ carbon of the ethyl substituent for ligand **30** resonates at 18.9 ppm.

Infrared (IR) Spectroscopy

Infrared spectra for thiosemicarbazones **30** – **34** were recorded as KBr pellets and selected IR absorptions are shown in Table 3.4.

Table 3.4.: Selected IR Absorptions for Ligands **30 - 34**

Ligand	N-H (cm^{-1})	C=N (cm^{-1})	C=S (cm^{-1})
30	3389[c], 3254[c], 3174[c]	1595[c]	1054[a]
31	3421[a], 3181[c], 3141[c]	1594[a]	1091[a]
32	3397[c], 3256[c], 3156[a]	1597[a]	1130[c]
33	3436[c], 3257[a], 3163[a]	1602[a]	1118[c]
34	3451[a], 3303[a], 3174[a]	1601[a]	1152[a]

[a] strong intensity; [b] broad; [c] medium intensity; [d] weak intensity

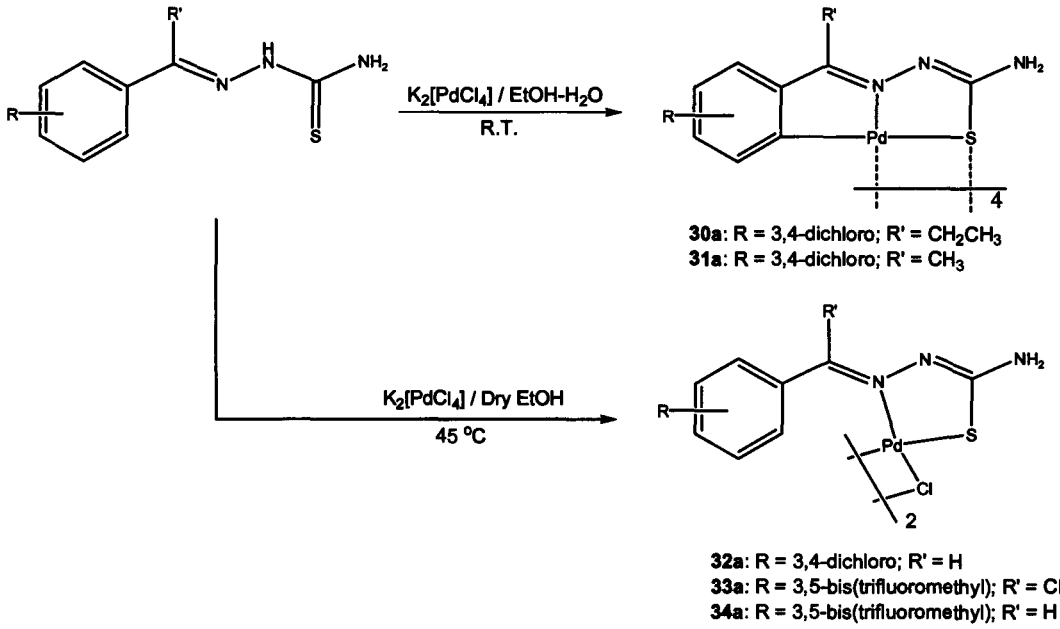
Three absorption bands are observed for the N-H vibrations in each ligand and is consistent with the three N-H bonds present.[12] The imine bond shows an absorption band in the expected region of the spectrum between 1590 and 1605 cm^{-1}. The C=S vibration for each thiosemicarbazone is assigned to a band observed between 1050 and 1155 cm^{-1}.

3.3. Preparation of Tetrameric and Dimeric Palladium(II) Complexes

3.3.1 General Methods

The cyclopalladation of 3,4-dichloropropiophenone and 3,4-dichloroacetophenone thiosemicarbazone (**30** and **31** respectively) was achieved by treatment of each ligand with a suspension of potassium tetrachloropalladate in deoxygenated ethanol

and water (Scheme 3.2.).[3] The reaction was stirred at ambient temperature under argon for 48 hours and the cyclopalladated tetrameric complexes (**30a** and **31a**) were isolated by vacuum filtration and washed with ethanol.



Scheme 3.2.

When this synthetic method was extended to ligands **32** – **34**, the expected tetrameric *ortho*-palladated complex was not isolated. Spectroscopic characterization of the crude solids isolated showed a mixture of possible decomposition products as well as unreacted ligand.

Initially, it was thought that the use of water either caused the cyclometallated product to decompose or hindered formation of the desired tridentate thiosemicarbazone Pd(II) complex. In addition, reaction at ambient temperature may have been too mild to allow C-H activation of the *ortho*-carbon. For these reasons, the synthetic method was modified (Scheme 3.2.). Thiosemicarbazones **32** – **34** were reacted with potassium tetrachloropalladate in dry, deoxygenated ethanol at 45 °C over 24 hours under argon. However, the complexes isolated are chloro-bridged dimers where the thiosemicarbazone ligand coordinates to palladium as a uninegative [N,S] bidentate donor (**32a** – **34a**). Quiroga *et al* have previously

reported the synthesis and characterization of thiosemicarbazone palladium(II) complexes of this type using a similar reaction method.[13] Hence, based on their structural characterizations as well as subsequent reactions discussed in Section 3.4., we believe the proposed structures of complexes **32a** – **34a** to be correct.

For the ligand 3,4-dichloro-benzaldehyde thiosemicarbazone (**32**), the reduced steric effect of the imine hydrogen may explain why C-H activation of the *ortho*-carbon does not occur. Lobana and co-workers found that cyclometallation of the aromatic ring only occurred when a bulky substituent, such as a methyl, was present on the imine carbon of the thiosemicarbazone ligand instead of hydrogen.[14] They rationalized that steric interaction between the bulky substituent and the aromatic ring pushed the ring closer to the metal thereby allowing cyclometallation to occur (Figure 3.2.). When the substituent is hydrogen, there is reduced steric interaction and the ring is not close enough to the metal for *ortho*-palladation to occur.

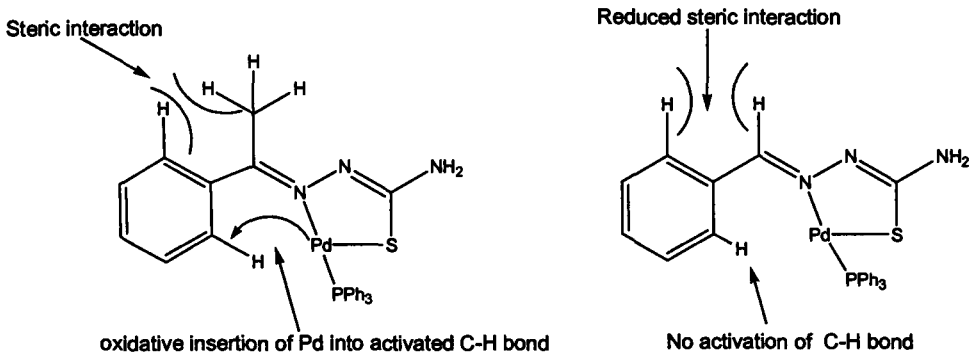


Figure 3.2.: Proposed rationalization for C-H activation of *ortho*-carbon in aryl thiosemicarbazone ligands[14]

This argument seems valid when the general mechanism of C-H activation is taken into account. Cyclopalladation via C-H activation is initiated by coordination of the nitrogen atom to the metal center. This is followed by the dissociation of a ligand from the metal complex to yield a highly reactive intermediate. This intermediate activates the C-H bond of the ligand in a kinetically controlled electrophilic step that shows a preference for the formation of a five-membered ring and attack on an aromatic ring thereby forming the cyclometallated product.[15]

In the case of ligands **33** and **34**, the presence of the bulky trifluoromethyl substituent in the 3 and 5 positions on the aromatic ring may hinder the oxidative insertion of palladium into the *ortho* C-H bond. It is also possible that the cyclometallated product may not be stable and decomposes before it is isolated, the strongly electron-withdrawing nature of the trifluoromethyl groups may weaken the *ortho* C-H bond making it unstable.

3.3.2. Physical Properties

The Pd(II) complexes **30a** – **34a** were isolated as yellow or orange solids in moderate to low yields (Table 3.5.). The tetrameric complexes **30a** and **31a** exhibit high thermal stability; only decomposing at temperatures greater than 300 °C.

Table 3.5.: Physical Appearance, Yields and Melting points for **30a – 34a**

Complex	Yield (%)	Physical Appearance	Melting Point
30a	15	Yellow Solid	decomp. 309-311 °C
31a	18	Yellow Solid	decomp. 307-309 °C
32a	67(crude)	orange solid	--
33a	48 (crude)	orange solid	--
34a	63 (crude)	orange solid	--

The crude dimeric complexes **32a** - **34a** were found to be insoluble in most organic solvents as well as water even at elevated temperatures, therefore purification by recrystallisation was not possible. Thin layer chromatography (TLC) was used to determine a suitable solvent system for separation and purification of the products. Satisfactory separation of the bands was not achieved and only a crude yield can be reported for these compounds. Complexes **32a** – **34a** were therefore used in subsequent reactions (Section 3.4.) without further purification.

3.3.3. Spectroscopic and Analytical Characterization

Complexes **30a** – **34a** are new compounds and were characterized using NMR and IR spectroscopies, mass spectrometry and elemental analysis (**30a** and **31a**). The complexes **33a** and **34a** were only characterized using proton and fluorine NMR and IR spectroscopy. The crude nature of these compounds meant that carbon-13 NMR spectra and elemental analysis could not be determined.

Nuclear Magnetic Resonance (NMR) Spectroscopy

Proton and carbon NMR spectra were recorded for complexes **30a** - **32a**. The crude nature as well as low solubility of the palladium(II) complexes **33a** and **34a** meant that the $^{13}C\{^1H\}$ NMR spectra obtained was unassignable and thus only their $^{19}F\{^1H\}$ and proton NMR data are reported. Table 3.6. lists the proton NMR data for complexes **30a** – **34a**.

For all of the complexes, the amino protons are observed between 7.05 and 7.30 ppm as a singlet. For complexes **30a** and **31a**, only two singlets were observed in the aromatic region and were integrated for one proton each. This supports palladation of the *ortho*-carbon upon loss of the proton. For **32a**, three protons are observed in the aromatic region, between 7.63 – 8.24 ppm, correlating to no C-H bond activation of the *ortho*-carbon.

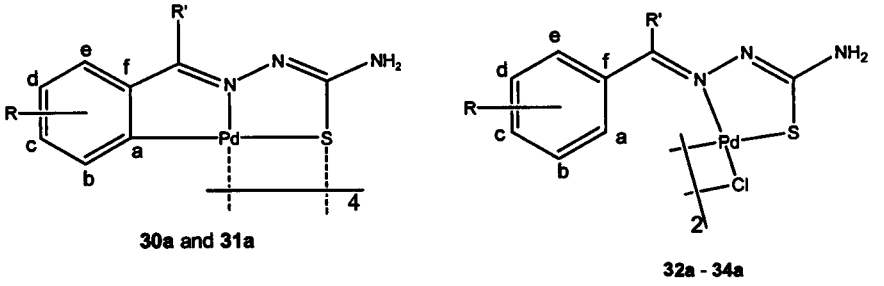


Figure 3.3. Structures of complexes **30a** – **34a**

Table 3.6.: ^{1}H and $^{19}F\{^{1}H\}$ NMR data for complexes **30a - 34a**

			^{1}H			^{19}F
Complex	**R**	**R'**	**Aromatic protons (ppm)**	**NH_2 (ppm)**	**Other (ppm)**	**CF_3 (ppm)**
30a	3,4-dichloro	Et	7.36 (s, H_b), 6.68 (s, H_e)	7.11	2.48 (CH_2), 0.925(CH_3)	--
31a	3,4-dichloro	Me	7.36 (s, H_b), 6.71 (s, H_e)	7.17	1.99 (CH_3)	--
32a	3,4-dichloro	H	8.24 (s, H_e), 7.91 (dd, $^{4}J(H_aH_e)$ = 1.86, $^{3}J(H_aH_b)$ = 8.53 Hz, H_a), 7.63 (d, $^{3}J(H_bH_a)$ = 8.50 Hz, H_b)	7.08	8.52 (H-C=N)	--
33a	3,5-bis(trifluoro-methyl)	Me	8.53 (s, H_a, H_e), 8.19 (s, H_c)	7.19	1.81 (CH_3)	-61.59
34a	3,5-bis(trifluoro-methyl)	H	8.71 (s, 2H, H_a, H_e), 8.12 (s, 1H, H_c)	7.31	8.45 (H-C=N)	-61.69

s = singlet, d = doublet, dd = doublet of doublets

The imine proton of **32a** is observed downfield, at 8.52 ppm, relative to its free ligand **32**. This deshielding effect is attributed to coordination of the imine nitrogen to palladium and supports the formation of an exo-cycle (where the imine functionality is not included within the chelate ring) between the ligand and the metal.[16] Studies of the effect of endo vs exo ring formation showed that when the imine functionality is included within the chelate ring (endo) there was an upfield shift of the imine proton; the opposite effect is observed for the exo-ring.[16-18] The imine proton of complex **34a** also exhibits this downfield shift compared to its free ligand (**34**).

The absence of a signal corresponding to the hydrazinic proton in the proton spectra for **30a – 34a** corroborates coordination of sulfur to palladium in the thiolato form and formation of a new imine bond upon deprotonation of the hydrazinic nitrogen.[2,13]

Figure 3.4. shows the proton and fluorine NMR spectra for complex **33a**. A less intense peak adjacent to each peak attributed to the major product is evident suggesting an isomeric mixture of compounds. Additionally, the fluorine NMR spectrum shows two singlets confirming the presence of another compound. A similar pattern was observed for **34a**. Separation of the mixture was not achieved

and so only the most prominent peaks were integrated and assigned, supporting the proposed dimeric structures of **33a** and **34a**.

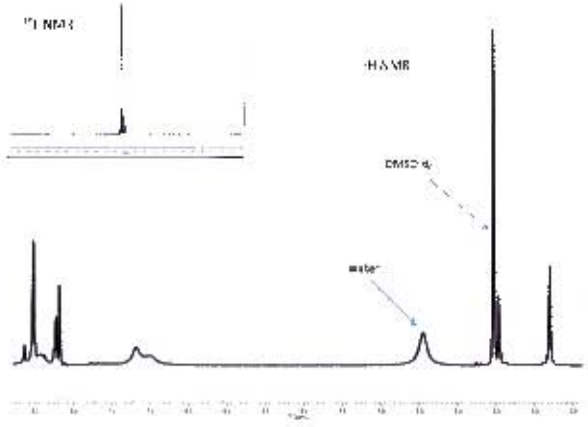


Figure 3.4.: ^{1}H and $^{19}F\{^{1}H\}$ (inset) NMR spectra for complex **33a**

The $^{13}C\{^{1}H\}$ NMR spectra were assigned only for complexes **30a** – **32a** and the data are summarized in Table 3.7. Complexes **33a** and **34a** were crude complexes and their carbon-13 NMR spectra could not be resolved.

Table 3.7.: $^{13}C\{^{1}H\}$ NMR Data for Complexes **30a** – **32a**

Complex	C=N (ppm)	C-S (ppm)	$C_{(aromatic)}$ (ppm)	Other (ppm)
30a	168.1	170.8	165.5 (C_a), 149.4 (C_f), 134.2 (C_c), 130.3 (C_d), 126.9 (C_e), 126.3 (C_b)	21.1 (CH_2), 12.0 (CH_3)
31a	166.1	167.7	165.1 (C_a), 150.7 (C_f), 133.9 (C_c), 129.9 (C_d), 126.8 (C_e), 126.6 (C_b)	14.3
32a	169.3	179.7	132.6 (C_c), 132.1 (C_e), 132.0 (C_h), 131.4 (C_d), 130.1 (C_a), 130.7 (C_f)	--

Coordination of the imine nitrogen to palladium is further supported by the assignment of the imine carbon for all complexes to a peak between 166.0 and 168.0 ppm, a downfield shift relative to the free ligands (**30** – **32**) where the C=N carbon peak occurs between 140.0 – 149.0 ppm. This is in accordance with resonances observed for similar complexes.[2,19] An upfield shift of approximately 10 ppm of the C-S carbon in the complex compared to the thione carbon of the free ligand confirms coordination of sulfur in the thiolato formation.[19]

For complexes **30a** and **31a**, the *ortho*-carbon shows a large shift from approximately 128.0 ppm in the free ligand to 165.0 ppm. This extreme deshielding

of the carbon nucleus is characteristic of palladation of the *ortho*-carbon and substantiates formation of the cyclopalladated complexes **30a** and **31a**.[17,19] A similar downfield shift of the *ortho*-carbon is not observed for **32a**, further evidence that C-H activation of the *ortho*-carbon did not occur. All other aromatic carbons are observed within the characteristic region of the spectrum for **30a – 32a**.

Infrared (IR) Spectroscopy

Table 3.8. shows selected IR absorption frequencies for complexes **30a – 34a**. Only two absorption bands are observed in the N-H region of the spectrum for all complexes, except for **32a** which shows a strong, broad band possibly due to overlapping of the two N-H vibrational bands, corresponding to loss of the hydrazinic proton and formation of an imine bond.

Table 3.8.: Selected IR absorptions for **30a – 34a**

Complex	N-H (cm^{-1})	C=N (cm^{-1})
30a	3459[c], 3376[c]	1595, 1561
31a	3442[c], 3369[c]	1597, 1560
32a	3436[a,b]	1615, 1560
33a	3409[c], 3308[c]	1614, 1579
34a	3469[a], 3279[a]	1618, 1582

[a] strong intensity; [b] broad; [c] medium intensity; [d] weak intensity

The formation of the second imine bond is further supported by the observation of two absorption bands between 1560 and 1620 cm^{-1}. The lower frequency band is assigned to the palladium coordinated imine nitrogen. A shift to lower frequency compared to the free ligand is attributed to the loss of double bond character upon coordination of the nitrogen to the metal. The higher frequency band is hence assigned to the stretching vibration of the newly formed C=N and points to the formation of the thiolate.

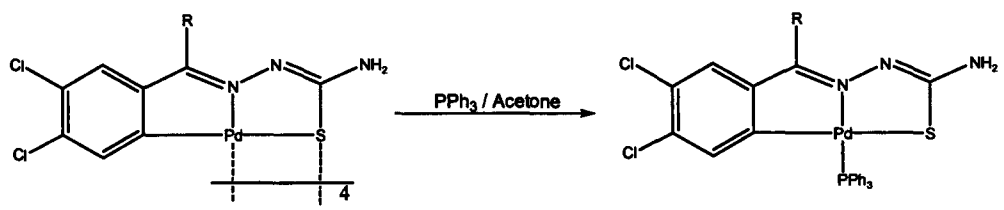
Assignment of the thiolate (C-S) bond vibration was not attempted as it occurs within a region of the spectrum (700-600 cm^{-1}) where several other bond vibrational bands are observed and thus could not be assigned with certainty.

3.4. Preparation of Monomeric Palladium(II) Complexes

3.4.1. General Methods

The bridging bonds linking each monomeric unit in the tetrameric (**30a** and **31a**) and dimeric (**32a** – **34a**) complexes were cleaved with triphenylphosphine using one of two methods. Reaction of **32a** - **34a** with triphenylphosphine was carried out despite the solids being a crude mixture as it was believed that if the chloro-bridged dimer was indeed present, it would be the only complex in the mixture to be cleaved with triphenylphosphine, hence only one product would be isolated.

The complexes **30a** and **31a** were suspended in dry, deoxygenated acetone and triphenylphosphine was added. The resulting mixture was stirred at room temperature for 4 hours; the products (**30b** and **31b**) were filtered and washed with cold acetone (Scheme 3.3.).

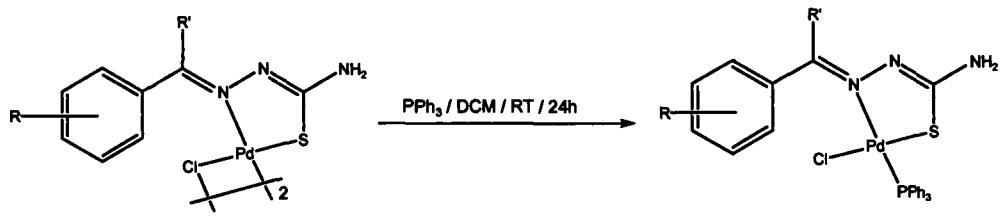


30a: R = CH_2CH_3
31a: R = CH_3

30b and 31b

Scheme 3.3.

Complexes **32a** – **34a** were suspended in dry, deoxygenated DCM and triphenylphosphine added (Scheme 3.4.). The reaction mixture was stirred for 24 hours at room temperature, filtered and the crude product was precipitated by addition of hexane. Recrystallisation of the crude products from a mixture of DCM and hexane gave the pure complexes **32b** – **34b**.



32a: R = 3,4-dichloro; R' = H
33a: R = 3,5-bis(trifluoromethyl); R' = CH_3
34a: R = 3,5-bis(trifluoromethyl); R' = H

32b - 33b

Scheme 3.4.

3.4.2. Physical Properties

Complexes **30b** – **34b** were isolated as yellow or orange solids. Table 3.9. summarizes the yields and melting points for these complexes.

Table 3.9.: Physical Appearance, Yields and Melting points for **30b – 34b**

Complex	Yield (%)	Physical Appearance	Melting Point
30b	72	Yellow Solid	280-283 °C
31b	75	Yellow Solid	decomp. 290-291 °C
32b	49	Orange solid	249-251 °C
33b	63	Orange solid	207-209 °C
34b	59	Orange solid	135-136 °C

In contrast to their dimeric starting complexes (**32a** – **34a**), complexes **32b** – **34b** exhibit good solubility in most organic solvents. Complexes **30b** and **31b** show the same limited solubility as their tetrameric analogues **30a** and **31a**.

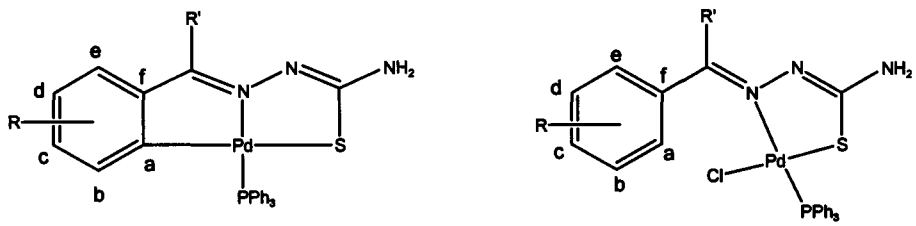
3.4.3. Spectroscopic and Analytical Characterization

Complexes **30b** – **34b** are new complexes and were fully characterized using NMR and IR spectroscopies, mass spectrometry and elemental analysis. NMR experiments were recorded in DMSO-d_6 for **30b** and **31b** and $CDCl_3$ for **32a** – **34b**; IR spectra were recorded as KBr pellets.

Nuclear Magnetic Resonance (NMR) Spectroscopy

Complexes **30b** – **34b** (Figure 3.6.) were characterized using proton, carbon, phosphorus and, in the case of **33b** and **34b**, fluorine NMR spectroscopy. Table 3.10. summarizes the ^{1}H, $^{31}P\{^{1}H\}$ and $^{19}F\{^{1}H\}$ NMR data and the ^{13}C NMR shifts are listed in Table 3.11.

For cyclopalladated complexes **30b** and **31b**, the amino (-N<u>H</u>$_2$) protons are observed in the proton NMR as a singlet at 6.88 and 6.79 ppm respectively, similar to previously synthesized cyclopalladated thiosemicarbazone phosphine complexes.[20] For the coordination complexes **32b** – **34b**, these protons are assigned to peaks observed further upfield between 4.77 – 5.01 ppm, in agreement with analogous [N,S] chelate Pd(II) thiosemicarbazone complexes.[21]



30b and 31b **32b - 34b**

Figure 3.6. Structures of monomeric complexes **30b** – **34b**

The protons for triphenylphosphine are observed in the expected region as broad multiplets. The peak for the imine proton of complex **32b** is observed as a doublet at 7.87 ppm with a coupling constant of 8.56 Hz, corresponding to long range proton to phosphorus coupling, as well as coordination of the triphenylphosphine co-ligand *trans* to the imine nitrogen.[22] Splitting of the imine proton for complex **34b** could not be ascertained as its peak overlaps with that of two aromatic protons of the thiosemicarbazone ligand.

Table 3.10.: ^{1}H, $^{31}P\{^{1}H\}$ and $^{19}F\{^{1}H\}$ NMR data for complexes **30b – 34b**

			^{31}P	^{1}H				^{19}F
Complex	**R**	**R'**	**(ppm)**	**H-C=N (ppm)**	**NH_2 (ppm)**	**Aromatic Protons (ppm)**	**Other (ppm)**	**CF_3 (ppm)**
30b	3,4-dichloro	Et	38.55	–	6.88	7.40-7.74 (m, PPh_3), 7.21 (s, H_b), 6.34 (s, H_e)	2.82 (CH_2), 1.11 (CH_3)	–
31b	3,4-dichloro	Me	38.75	–	6.79	7.42-7.64 (m, PPh_3), 7.20 (s, H_b), 6.10 (s, H_e)	2.76 (CH_3)	–
32b	3,4-dichloro	H	27.10	7.87	4.93	8.47 (m, H_b, H_e), 7.70-7.80 (m, PPh_3), 7.40-7.55 (m, PPh_3, H_a)	–	–
33b	3,5-bis(trifluoro-methyl)	Me	29.22	–	4.77	8.19 (s, 2H, H_a, H_e), 7.93 (s, 1H, H_c), 7.46-7.63 (m, 6H, PPh_3), 7.36-7.45 (m, 9H, PPh_3)	2.52 (CH_3)	-62.93
34b	3,5-bis(trifluoro-methyl)	H	27.74	8.69	5.01	8.66 (s, 2H, H_a, H_e), 7.89 (s, 1H, H_c), 7.74-7.80 (m, 6H, PPh_3), 7.45-7.54 (m, 9H, PPh_3)	–	-63.38

s = singlet, d = doublet, dd = doublet of doublets and m = multiplet

The $^{31}P\{^{1}H\}$ NMR spectrum shows a singlet at *ca.* 38.50 ppm for complexes **30b** and **31b** and a singlet between 27.00 and 29.20 ppm for complexes **32b** – **34b**. These resonances agree with those of similar complexes and further support coordination of phosphorus *trans* to the imine nitrogen.[2,18,22]

Cleavage of the Pd-$S_{bridging}$ bonds in **30a** and **31a** by triphenylphosphine to yield complexes **30b** and **31b** results in a downfield shift for the signal due to the thiol carbon to approximately 176.0 ppm. The alkyl carbons of the R' group occur slightly upfield compared to the tetrameric analogues (**30a** and **31a**).

Table 3.11.: $^{13}C\{^1H\}$ NMR Data for Complexes **30b – 34b**

Complex	C=N (ppm)	C-S (ppm)	$C_{(aromatic)}$ (ppm)	Other (ppm)
30b	168.2	176.9	164.1 (C_a), 151.5 (C_f), 136.5 (C_d), 133.9 – 128.6 (C_c and PPh_3), 126.3 (C_e), 125.9 (C_b)	19.5 (($\underline{C}H_2$), 10.9 ($\underline{C}H_3$)
31b	163.6	176.6	152.7 (C_a), 136.1 (C_f), 133.8 (C_d), 133.7 (C_c), 128.5-131.1 (PPh_3), 126.2 (C_e), 126.1 (C_b)	13.0 (CH_3)
32b	150.9	175.8	128.3-134.6 (C_a, C_b, C_c, C_d, C_e, C_f and PPh_3)	–
33b	165.7	172.1	142.3 (C_f), 128.0-134.1 (C_a, C_b, C_c, C_d, C_e and PPh_3)	123.1 (CF_3), 21.8 (CH_3)
34b	149.6	177.0	128.4-134.6 (C_a, C_b, C_c, C_d, C_e, C_f and PPh_3)	123.7 (CF_3)

In **30b** the imine carbon resonates at a similar shift to **30a** and is slightly more shielded in complex **31b** compared to **31a**. For complexes **32b** – **34b**, the thiol carbon is observed between 172.0 – 177.0 ppm. The imine carbon for **32b** and **34b**, where the R' substituent is a hydrogen, resonate at a similar shift which is upfield from that observed for **33b**, where the R' substituent is a methyl group which may result in deshielding of the imine carbon. For all of the complexes, the aromatic carbons of the thiosemicarbazone and the triphenylphosphine ligands are observed in the expected region, between 126.0 and 165.0 ppm.

Infrared (IR) Spectroscopy

Selected infrared absorption bands for **30b** – **34b** are listed below in Table 3.12.

Table 3.12: Selected IR Absorption Bands for **30b – 34b**

Complex	N-H (cm^{-1})	C=N (cm^{-1})
30b	3466, 3289	1618, 1577
31b	3474, 3332	1601, 1579
32b	3468, 3365	1595, 1581
33b	3446	1624, 1603
34b	3461, 3341	1607, 1577

Absorption bands similar to that of their multimeric analogues (**30a** – **34a**, Table 3.8.) are observed for the C=N and N-H vibrations in **30b** – **34b**. As with complexes **30a** – **34a**, the low frequency band observed for the imine vibrations is assigned to the C=N bond coordinated to palladium. For complex **33b**, a broad absorption band,

possibly due to overlapping of the N-H vibrations is observed for the two N-H bonds at 3446 cm^{-1}.

Mass Spectrometry and Elemental Analysis

The tetrameric complexes **30a** and **31a** as well as complexes **30b** – **34b** were further characterized using ESI-mass spectrometry and elemental analysis. Table 3.13. lists the fragments corresponding to the proposed molecular formulae for these compounds.

Table 3.13. Mass Spectral Results for Complexes **30a** – **34b**

Complex	Calculated Molar Mass (g/mol)	Molecular Fragment	Assignment
30a	1522.36	1522	$[M]^+$
31a	1466.24	1468	$[M + 2H]^{2+}$
30b	642.88	644	$[M + H]^+$
31b	628.85	630	$[M + H]^+$
32b	651.28	615.95	$[M - Cl]^+$
33b	732.42	695.98	$[M - Cl]^+$
34b	718.98	681.99	$[M - Cl]^+$

The C, H, N and S values found for these compounds are within acceptable limits compared to their calculated values.

3.5. X-ray Crystallography

Single crystals were grown by slow evaporation of DMSO from the NMR sample for complex **30b** and from a DCM-hexane mixture for complex **32b**. Their molecular structures were elucidated using single crystal X-ray diffraction and validate the spectroscopic and analytical characterization of the cyclopalladated (**30b** and **31b**) and the bis-chelated (**32b** – **34b**) thiosemicarbazone palladium(II) complexes. The molecular structure of **30b** is shown in Figure 3.7. and **32b** in Figure 3.8. Table 3.14. lists the crystallographic data for both molecular structures. Selected bond lengths and angles are given in Table 3.15.

Complex **30b** crystallizes with an orthorhombic system and space group $Pca2_1$. In the crystal system, the terminal amine of one complex molecule intermolecularly hydrogen bonds to the oxygen of two solvent molecules.

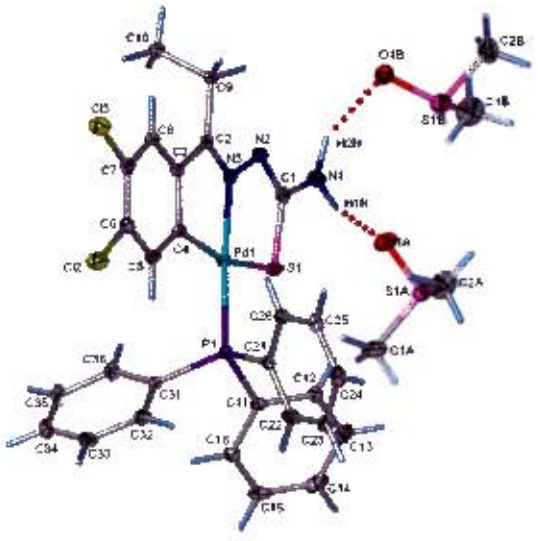


Figure 3.7.: Molecular structure of **30b** with ellipsoidal model of probability level = 40%. The hydrogen bonds are shown as dotted lines

Table 3.14.: Crystallographic Data for complexes **30b** and **32b**

	30b	**32b**
Empirical formula	$C_{32}H_{35}Cl_2N_3O_2PPdS_3$	$C_{26}H_{21}Cl_3N_3PPdS$
Empirical Weight	799.09	651.24
Crystal size	0.16 x 0.14 x 0.11 mm	0.12 x 0.11 x 0.09 mm
Crystal system, space group	Orthorhombic, $Pca2_1$	Monoclinic, $P2_1/c$
a	15.6030(6) Å	17.0141(5) Å
b	9.6129(2) Å	20.0919(6) Å
c	23.0082(4) Å	17.4680(4) Å
alpha	90 deg.	90 deg
beta	90 deg.	118.139(2) deg.
gamma	90 deg.	90 deg
Volume	3451.00(16) Å^3	5265.6(3) Å^3
Z	4	8
Calculated density	1.538 Mg/m^3	1.643 Mg/m^3
F(000)	1632	2608
R indices (all data)	R1 = 0.0417, wR2 = 0.0677	R1 = 0.0883, wR2 = 0.1153

The thiosemicarbazone ligand bonds to the palladium(II) atom in the expected [C,N,S] fashion forming two five-membered chelate rings.[2,3] The fourth coordination site on the metal is occupied by a triphenylphosphine ligand. Inspection of the angles formed between the metal and the coordinated atoms show that the metal is contained within a slightly distorted square-planar environment. The angles N(3)-

Pd(1)-S(1) 82.48(8) and N(3)-Pd(1)-C(4) 81.30(12) formed between the thiosemicarbazone ligand and the metal are less than 90°. The angles P(1)-Pd(1)-S(1) 99.85(3) and P(1)-Pd(1)-C(4) 96.55(10) are therefore greater than 90°. Collectively the bite angles observed in the molecular structure compare favourably with those of analogous cyclopalladated triphenylphosphine complexes.[2,3]

The bond lengths between palladium and the donor atoms are within the range of 2.03 and 2.33 Å with the shorter bond distances occurring between the metal and the N(3) and C(4) atoms. The strong *trans* influence of the phosphorus atom is reflected in the Pd(1)-N(3) bond distance which is slightly longer than the expected length calculated from the covalent radii of palladium and nitrogen (2.01 Å).[2,3,23]A C(1)-S(1) bond length of 1.762(4) is consistent with single bond character and confirms that sulfur bonds to the metal in the thiolate form, additionally the C(1)-N(2) bond length substantiates the formation of a double bond upon deprotonation of the hydrazinic nitrogen.[2,3]

Table 3.14.: Selected Bond Lengths and Angles for Complexes **30b** and **32b**

	Complex 30b	**Complex 32b**
Pd(1)-S(1)	2.3324(9) Å	2.2403(14) Å
Pd(1)-P(1)	2.2614(9) Å	2.2487(14) Å
Pd(1)-N(3)	2.036(3) Å	2.111(4) Å
Pd(1)-C(4)	2.038(4) Å	–
Pd(1)-Cl(1)	–	2.3447(14) Å
C(1)-S(1)	1.762(4) Å	1.746(6) Å
C(1)-N(2)	1.324(5) Å	1.319(7) Å
C(2)-N(3)	1.299(4) Å	1.289(7) Å
N(3)-Pd(1)-S(1)	82.48(8) °	83.37(12) °
N(3)-Pd(1)-C(4)	81.30(12) °	–
N(3)-Pd(1)-Cl(1)	–	95.90(12) °
P(1)-Pd(1)-S(1)	99.85(3) °	92.11(5) °
P(1)-Pd(1)-C(4)	96.55(10) °	–
P(1)-Pd(1)-Cl(1)	–	88.63(5) °

Figure 3.8. shows the molecular structure of complex **32b**. The complex crystallizes with a monoclinic $P2_1/c$ system. There are two complex molecules per asymmetric unit.

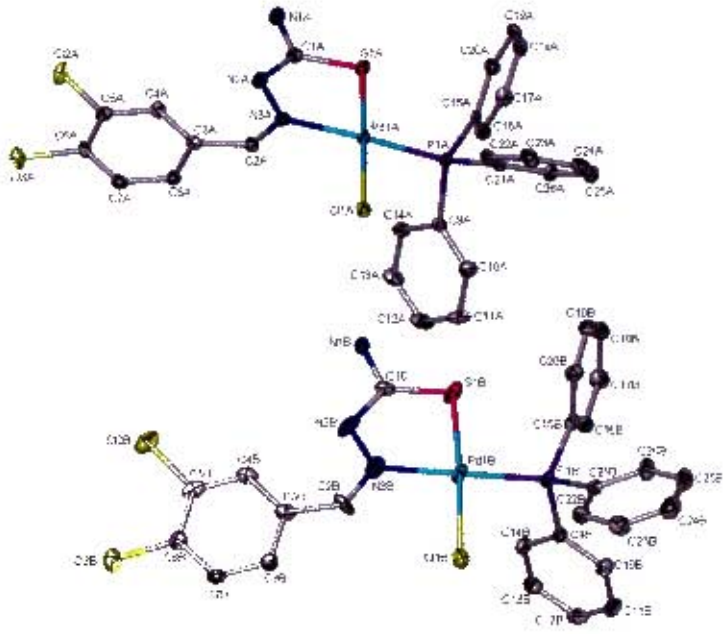


Figure 3.8. Molecular structure of **32b** showing the asymmetric unit with atomic labelling scheme. Ellipsoidal models were draw at 40% probability level. All hydrogen atoms are omitted for clarity.

The thiosemicarbazone ligand (**32**) coordinates to palladium via the imine nitrogen and thiolato sulfur forming a five membered chelate ring. The other two coordination sites on palladium are occupied by a chlorido ligand and triphenylphoshine; the triphenylphosphine ligand is coordinated *trans* to the imine nitrogen. Inspection of the bond angles formed between the donor atoms and palladium correspond to a slightly distorted square-planar environment around the metal.

The Pd(1)-S(1) (2.2403(14) Å), Pd(1)-P(1) (2.2487(14) Å), Pd(1)-N(3) (2.111(4) Å) and Pd(1)-Cl(1) (2.3447(14) Å) bond distances are analogous to complexes of this type reported in the literature.[21,24] The value observed for the C(1)-S(1) bond distance (1.746(6) Å) is closer to the bond length expected for a C-S single bond (1.82 Å) than that of a normal C=S double bond (1.56 Å),[25] corroborating that sulfur coordinates to palladium in the thiolato form.[26] The C(1)-N(2) and C(2)-N(3) bond distances of the complex are similar indicating increased double bond character for the C(1)-N(2) bond supporting formation of a new imine bond in the thiosemicarbazone ligand upon coordination of sulfur to palladium.[27-29]

The bite angles P(1)-Pd(1)-S(1) and P(1)-Pd(1)-Cl(1) (92.11(5)° and 88.63(5)° respectively) show the least deviation from 90°, thus the greastest deviation is observed for the N(3)-Pd(1)-S(1) and N(3)-Pd(1)-Cl(1) bite angles that of 83.37(12) ° and 95.90(12) ° respectively. Overall, the bond angles observed are comparable to literature examples of these types of bidentate thiosemicarbazone Pd(II) complexes.[21,24]

3.6. Conclusion

The complexation of thiosemicarbazone ligands **30** – **34** have shown that the position of the substituents on the aryl-ring and the size of the substituent present on the imine carbon influence the bonding mode of aryl-thiosemicarbazones to palladium.

Ligands **30** and **31**, where the imine substituent is an alkyl group (Me, Et), form tridentate cyclopalladated tetrameric complexes (**30a** and **31a**) when reacted with $K_2[PdCl_4]$ while bidentate chloro-bridged dimers (**32a**) is isolated for the 3,4-dichloro-benzaldehyde thiosemicarbazone derivative (**32**).

For thiosemicarbazones **33** and **34**, reaction with the palladate salt yields a mixture of products (possibly isomers) as observed in the proton NMR, and the position and size of the aryl substituents may hinder C-H activation of the ligands.

Cleavage of the Pd-$S_{bridging}$ (**30a** and **31a**) and the Pd-$Cl_{bridging}$ (**32a** – **34a**) bonds was achieved by reaction of the corresponding tetramer or dimer with triphenylphosphine yielding monomeric complexes (**30b** – **34b**).The molecular structures of **30b** and **32b** were determined using X-ray diffraction and confirm the solid state structures of all of the mononuclear complexes synthesized.

3.7. References

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Chapter 4: Biological and Catalytic Activity of Thiosemicarbazone Ligands and their Palladium(II) Complexes

4.1. Biological Studies

The metal chelating abilities of thiosemicarbazones are believed to partially account for their biological activity. These compounds contain several donor atoms that could chelate to a metal within the cell giving rise to the actual active species. In addition, several other factors have contributed to the steady rise in biological research of their metal complexes. The bioactivities of thiosemicarbazones may be beneficially altered upon coordination to a metal.[1] The lipophilic nature of the complex might be enhanced compared to the free ligand and certain long term side effects may decrease or may be altogether avoided since metal complexes can break down and the metal ion can then interact with the organism.[2-4] In addition, the metal complex could exhibit bioactivities not shown by the free thiosemicarbazone ligand and coordination could yield a significant reduction in drug resistance.[1]

As discussed in Chapter 1 (Section 1.2.1.), the high toxicity of cisplatin led to the investigation of other platinum complexes which exhibit the same beneficial properties but are less toxic. Carboplatin ([diamine(cyclobutane-1,1-carboxylato)platinum(II)]), one of the second generation platinum(II) drugs discovered, has shown activities equivalent to cisplatin but with a markedly lower toxicity.[5,6] As part of this search for improved antitumoral drugs relative to cisplatin, palladium(II) complexes were also amongst the first to be used in clinical trials since they exhibit similar aqueous chemistry to those of platinum(II).[7] However, palladium(II) complexes are generally thermodynamically and kinetically less stable than Pt(II) complexes and this accounted for the lower activity and higher toxicity of complexes *cis*-$Pd(NH_3)Cl_2$ and $Pd(1,2\text{-diaminocyclohexane})_2Cl_2$ compared to their platinum analogues.[7-9]

Since these first discoveries, the preparation of Pd(II) complexes with inert ligands such as sulfur donors grew in importance after it was suggested that N,S chelates of Pd(II) are better antitumor agents than those of other metals. The N,S chelate complexes of other metals do not have sufficient thermodynamic stability while platinum(II) N,S chelates are kinetically inert. In contrast, palladium(II) chelates possess the proper lability to transport the metal to DNA, allowing it to interact with it.[10]

In this regard, thiosemicarbazones have arisen as an excellent class of N,S donor ligands in the search for better antitumoral palladium(II) complexes. Thiosemicarbazones display anticancer activity and it is believed that their mechanism of action is through the inhibition of ribonucleotide reductase.[11] Their use in conjunction with Pd(II), which is known to damage DNA, produces a synergistic effect which could potentially lead to the development of more effective cancer chemotherapies. Thus, the study of Pd(II) thiosemicarbazone complexes as antiproliferative agents has emerged as an area of great importance.

Pd(II) complexes of aryl derived thiosemicarbazones have been tested for antitumoral and anticancer activity against several cancer cell lines including, human and murine tumor resistant cell lines that are resistant and sensitive to cisplatin,[12-14] human breast cancer [15] and bladder cancer.[15] In certain cases, the Pd(II) complexes were found to be better cytotoxic agents than cisplatin as well as their free ligands. A tridentate phenanthrenequinone thiosemicarbazone Pd(II) complex was found to exhibit superior selectivity toward breast cancer tumor cells which have previously shown resistance to conventional chemotherapies.[16]

The antiplasmodial activity of thiosemicarbazones against *Plasmodium falciparum* strains has been reported.[17-22] These compounds are believed to affect processes associated with haemoglobin (Hb) digestion in the food vacuole of the parasite through several possible inhibitory mechanisms of action. The thiosemicarbazone could act as a metal chelator that coordinates via its donor atoms to endogenous metals such as Fe(III), which is critical for the biological activity of a number of

plasmodial proteins, thus inhibiting the growth of the malaria parasite by withholding it from metal dependent enzymes such as cysteine and aspartic proteases.[20,22] Secondly, they could co-bind metals and substrate recognition sites consequently enhancing the natural inhibitory effects of endogenous metals on protease catalytic sites.[23]

Thirdly, thiosemicarbazones are believed to inhibit cysteine proteases within the malaria parasite through covalent modification of the cysteine thiol groups either via the electophilic centers (thione carbon and/or imine carbon) of the thiosemicarbazone moiety. The main function of *Plasmodium* cysteine proteases is haemoglobin (Hb) hydrolysis which, provides amino acids for parasite protein synthesis,[24,25] provides space for the growing parasite [26] and sustains the osmotic stability of malaria parasites.[27] Thus modification of the cysteine thiol within the active site needed for hydrolysis inhibits the enzyme.

The use of thiosemicarbazone metal complexes as antimalarial agents is sparse. Copper(II), nickel(II) and iron(II) complexes of 2-acetyl pyridine derived thiosemicarbazones have been screened for antimalarial activity.[28] The Cu(II) and Fe(II) complexes were found to exhibit modest activity compared to their free ligands while the Ni(II) complexes showed no activity. *The investigation of thiosemicarbazone Pd(II) complexes as antimalarial agents has not been reported in the literature.*

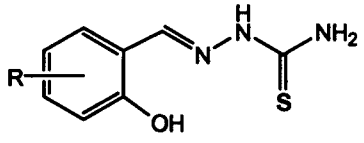
A preliminary biological study of selected thiosemicarbazones prepared (**26** – **31**) and their corresponding palladium(II) complexes (**26a** – **29a**, **30b** and **31b**) was undertaken. These compounds were tested *in vitro* for activity against several cancer cell lines and two strains of *P. falciparum*. In addition, apoptosis experiments were carried out for ligand **27**.

4.1.1. Anticancer Activity

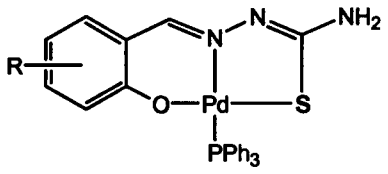
The salicylaldehyde derived thiosemicarbazones ligands (**26** – **29**) and their corresponding Pd(II) complexes of type (salicylaldiminato thiosemicarbazone)Pd(PPh_3) (**26a** - **29a**) were evaluated *in vitro* for activity as

anticancer agents. All of the compounds were first screened for activity against the cancer cell line WHCO1, an oesophageal cancer cell line of South African origin with Doxorubicin as the control drug (Table 4.1.).

The compounds showing the best activity were then screened against several other cell lines. Figure 4.1. shows the structures of the ligands and palladium(II) complexes tested. Activities for these compounds are expressed as IC_{50}, the compound concentration required for 50 % inhibition *in vitro*. The ligand, 3-^{t}butyl-2-hydroxy benzaldehyde thiosemicarbazone (**27**), showed the best activity (IC_{50} = 1.10 μM) out of all compounds screened against the WHCO1 cell line. However, its corresponding complex (**27a**) exhibited moderate to weak cytoxicity (IC_{50} = 54.38 μM).



26: R = 5-Chloro
27: R = 3-tert. Butyl
28: R = 3-Methoxy
29: R = H



26a: R = 5-Chloro
27a: R = 3-^{t}Butyl
28a: R = 3-Methoxy
29a: R = H

Figure 4.1. Structures of thiosemicarbazones and their corresponding palladium(II) complexes tested for anticancer activity

Similarly, ligand **26** shows better activity than its corresponding complex (**26a**). The opposite effect is observed for compound **28**, its complex (**28a**) impressively showed a value almost 40 times lower than its IC_{50} value of 95.13 μM. In the case of complex **28a**, this difference in activity may be due to one of two factors; coordination of the thiosemicarbazone to palladium may alter its lipophilic character thus increasing activity or it may be a synergistic effect, the ligand dissociates within the cell and interacts with the ribonucleotide reductase enzyme while the free metal ion interacts with DNA.[15,29]

Table 4.1. IC_{50}[a] Values for Compounds **26 – 29a** Against the WHCO1 Cell Line

Compound	IC_{50} [μM]	95% Confidence Interval
26	10.83	9.31 – 12.59
27	1.10	0.91 – 1.28
28	95.13	71.61-126.40
29	–[b]	--
26a	24.00	19.27 – 29.90
27a	54.38	15.70 – 188.3
28a	2.56	2.33 -2.82
29a	6.68	6.35 – 7.03
Doxorubucin	0.58	0.48-0.70

[a] IC_{50}: compound concentration causing 50 % inhibition *in vitro;* [b] --: inactive at the highest concentration tested

Interestingly, ligand **29** was not active at the maximum concentration used while its palladium complex (**29a**) showed good activity; coordination of thiosemicarbazone **29** to palladium clearly modifies its cytotoxic properties most likely by increasing its lipophilic nature. Out of all the Pd(II) complexes tested, complex **28a** shows the greatest cytotoxic activity with an IC_{50} value of 2.56 μM.

Inspection of the results obtained for the free ligands (**26 – 29**) shows that having a substituent on the aryl ring increases the activity of the compound however; the degree of activation may be dependent on the position of the substituent as well as the inductive effect of the substituent. Compound **28** has a strongly electron donating substituent in position 3 on the ring yet it does not show any appreciable activity while thiosemicarbazone **27**, where the tertiary butyl group donates electron density into the ring to a lesser extent than **28**, shows the best activity. Compound **26** has an electron-withdrawing chloro-substituent on the ring and it exhibits intermediate activity; better than **28**. This may be explained by the fact that the chloro group is in position 5 on the ring. It is possible that the position as well as the electronic nature of the aromatic substitutent may influence the way the ligand binds within the enzyme pocket. Further studies regarding the structure-activity relationship of these compounds need to be undertaken in order to establish the actual consequence of the aromatic substituent. These observations however, cannot be extended to their

corresponding complexes which do not show a trend regarding the substituents on the ring.

In a previous study, cisplatin has shown an IC_{50} value of approximately 13 μM against the WHCO1 cell line.[30] The free ligands **26** and **27** as well as complexes **28a** and **29a** all exhibit cytotoxicities lower than this value against this cell line. An actual comparison to cisplatin is not possible as these compounds are neither structurally similar to cisplatin nor were they screened with cisplatin in the same experiment. Nevertheless, it would be interesting to investigate the activity of these compounds (**26**, **27**, **28a** and **29a**) *in vitro* together with clinically used drugs in order to ascertain if they are more potent inhibitors.

The three compounds showing the best activities, ligand **27** and palladium complexes **28a** and **29a**, were then screened against two additional oesophageal cancer cell lines of South African origin (WHCO5 and WHCO6), five oesophageal cancer cell lines of Japanese origin (KYSE30, KYSE70, KYSE180, KYSE410 and KYSE450) and two cervical cancer cell lines (CaSki and HeLa). The results are shown in Table 4.2. along with their activity against WHCO1.

Table 4.2. IC_{50} [a] Values for Compounds **27**, **28a** and **29a** Against Several Cancer Cell Lines

	Ligand 27		Complex 28a		Complex 29a	
Cell Line	**IC_{50} [μM]**	**95% Confidence Interval**	**IC_{50} [μM]**	**95% Confidence Interval**	**IC_{50} [μM]**	**95% Confidence Interval**
WHCO1	1.10	0.91 – 1.28	2.56	2.33 -2.82	6.68	6.35 – 7.03
WHCO5	4.03	3.47-4.69	2.11	0.69-6.39	n/d	n/d
WHCO6	6.32	5.05-7.92	12.23	5.40-27.70	43.13	15.00-124.00
KYSE30	3.02	2.21-4.13	1.48	1.11-1.96	3.76	2.63-5.39
KYSE70	7.98	6.66-9.56	-- [b]	--	10.28	8.14-12.97
KYSE180	7.27	6.46-8.19	5.23	--	6.43	5.40-7.65
KYSE410	7.46	6.48-8.59	46.02	23.51-90.11	--	--
KYSE450	1.64	1.33-2.02	2.19	1.42-3.36	4.11	2.86-5.91
CaSki	13.03	9.56-17.75	--	--	--	--
HeLa	0.91	0.79-1.06	--	--	70.61	61.81-80.67

[a] IC_{50}: compound concentration causing 50 % inhibition *in vitro*; [b] --: inactive at the highest concentration tested

The free thiosemicarbazone ligand **27**, showed good activity against all of the cell lines screened with the best activity observed in the cervical cancer cell line HeLa. Complex **28a** exhibited good IC_{50} values against cell lines WHCO1, WHCO5, KYSE30, KYSE180 and KYSE450; intermediate activity against WHCO6 and negligible activity against KYSE410 cell lines. It did not show any activity against the remaining cell lines at the highest concentrations used (200 μM). Complex **29a** displayed good activity in only four of the oesophageal cancer cell lines tested; was not active in the CaSki line and showed negligible activity against the HeLa cell line.

Complex **29a** and similar [O,N,S] tridentate thiosemicarbazone Pd(II) analogues have been previously screened for *in vitro* anticancer activity against the promyelocytic HL-60 and histiocytic lymphoma U-937 cell lines.[31] Most of these complexes exhibited IC_{50} values less than 10 μM. In addition, they were found to be more potent cytotoxic agents than the human clinical drugs cisplatin, BCNU (1,3-bis(2-chloroethyl)-*l*-nitrosourea), hydroxyurea and 5-FU (5-fluorouracil) which were also screened during the same experiments.[31] Similarly, complex **29a** has displayed activities lower than 10 μM in four of the oesophageal cell lines tested.

In the WHCO5 cancer cell line, complex **28a** shows better activity (IC_{50} = 2.11 μM) than compound **27** (IC_{50} = 4.03 μM) and this is also observed for the KYSE30 and KYSE180 cell lines. Complex **29a** demonstrated an IC_{50} value comparable to ligand **27** (IC_{50} = 3.02 μM) but was almost 2.5 times less active than **28a** against KYSE30. Both complexes are more active than **27** against KYSE180 while neither complex exhibits activity against the CaSki line. Overall, thiosemicarbazone **27** was found to be a good cytotoxic agent, it consistently displayed good activity against all cell lines tested while the complexes **28a** and **29a** only exhibited cytotoxicity against selected cell lines.

Due to the favourable IC_{50} values observed for ligand **27** against almost all cell lines treated, dose-response curves were determined for ligand **27** and its corresponding Pd(II) complex **27a** in the HeLa and WHCO1 cell lines, the two cancer cell lines where ligand **27** displayed its lowest IC_{50} values (0.91 and 1.10 μM respectively). Complex **27a** displayed poor cytotoxicity during the initial screening of the

compounds **26** – **29a** against WHCO1, therefore its dose-response curves were determined merely for comparison purposes. In addition, apoptosis experiments for these cell lines were carried out using ligand **27**. Figure 4.2. shows the dose-response curves ascertained for **27** and **27a** against the HeLa and WHCO1 cell lines.

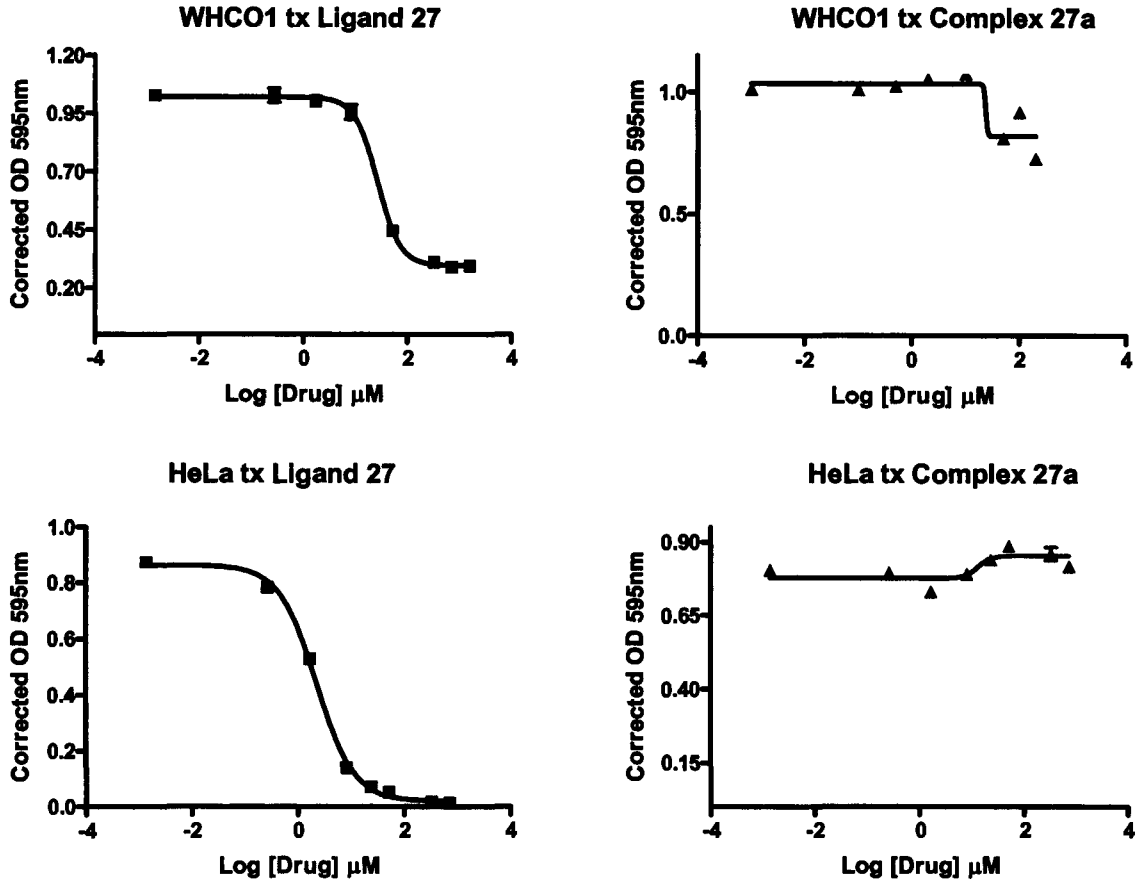
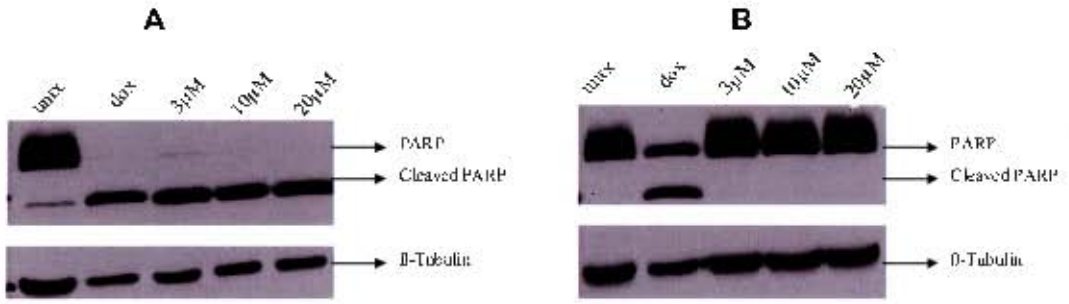


Figure 4.2. Dose-response curves for **27** and **27a** against the HeLa (below) and WHCO1 (above) cell lines.

WHCO1 cells treated with ligand **27** gave an IC_{50} of 5.52 µM (4.72-6.46 µM confidence interval) and complex **27a** was inactive against WHCO1 cells as treatment at the highest concentration did not kill cells. HeLa cells were more sensitive to **27** than WHCO1 cells with an IC_{50} of 0.64 µM (0.60-0.70 µM confidence interval). An IC_{50} value could not be obtained for the treatment of HeLa cells with **27a** as the compound did not affect the proliferation of the cells. It is evident that ligand **27** is very active against HeLa cells and that it does affect cell proliferation in

WHCO1 cells. The metal complex **27a** was completely inactive against both HeLa and WHCO1 cell lines, this could be a result of poor solubility of the compound.

PARP (Poly Adenosine-Diphosphate Ribose Polymerase) cleavage was investigated by Western blot analysis in order to determine the mode of cell death induced by ligand **27**. Figure 4.3. shows the western blot diagrams for this experiment.



Legend:
A: HeLa cells treated with **27**
B: WHCO1 cells treated with **27**
Untx: untreated cells: negative control
Dox: doxorubicin-treated cells: postitive control

Figure 4.3. Western Blot diagrams for HeLa and WHCO1 cells treated with ligand **27**

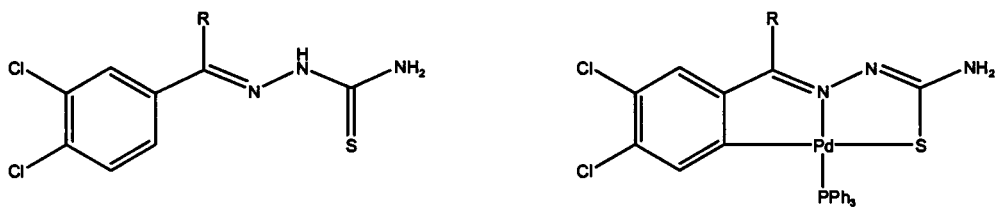
PARP is a known caspase substrate and cleavage of PARP into two distinct fragments (116kDa and 85kDa) serves as a marker of apoptosis. Apoptosis is the process of programmed cell death which involves a series of biochemical steps resulting in morphological changes to the cell membrane including cell shrinkage, nuclear fragmentation and chromosomal DNA fragmentation.[32] HeLa cells treated with three concentrations of thiosemicarbazone **27** showed considerable cleavage of PARP whereas WHCO1 cells treated with this compound showed no PARP cleavage. Treatment of cells with 5 μM doxorubicin served as a positive control.

From these results it is evident that **27** kills HeLa cells via apoptosis. The mode of cell death for WHCO1 cells at the above treatment concentrations of **27** is clearly not apoptosis and it might be interesting to investigate whether **27** triggers cell death in WHCO1 cells by necrosis (premature death of cells usually induced by disruption of cell membrane) [33], senescence (loss of cell's ability to divide due to DNA double

strand breaks or toxins) or autophagy (degradation of a cell by separating the contents from the rest of the cytoplasm).[34]

4.1.2. Antimalarial Activity

The compounds tested for anticancer activity, thiosemicarbazones **26** – **29** and Pd(II) complexes **26a** – **29a**, as well as ligands **30** and **31** (Figure 4.4.) and their cyclopalladated complexes (**30b** and **31b**) were evaluated for antimalarial activity *in vitro* against the *P. falciparum* strains, W2 (chloroquine resistant) and D10 (chloroquine sensitive) and the data are listed in Table 4.3. The control drugs used in the experiment were chloroquine (CQ) and artemisinin (ART) for the W2 strain and chloroquine for the D10 strain.



30a: R = CH_2CH_3
31a: R = CH3

30b: R = CH_2CH_3
31b: R = CH_3

Figure 4.4. Structures of thiosemicarbazones **30** and **31** and their corresponding complexes, **30b** and **31b**

For the D10 strain, the compounds were first screened for percentage parasite survival at a single concentration in order to determine the most active compounds for which, IC_{50} values were ascertained. All of the ligands (**26** - **31**) as well as complex **31b** showed cell survival close to or greater than 50 %. The lowest percentage cell survival was observed for complex **29a** (27 %) with complexes **26a**, **28a** and **30b** displaying percentage survivals between 31 and 38 %.

From the IC_{50} values obtained for these complexes against the D10 strain, complexes **26a** and **30b** exhibit good activity; complexes **28a** and **29a** also show good activity. It is interesting to note that the two complexes showing the best activities against this strain have chloro (**26a**) and dichloro (**30b**) substituents on the

aryl ring. Complex **29a**, the unsubstituted palladium(II) salicylaldimine thiosemicarbazone, is less active than complex **28a**, where the aryl substituent is the electron-donating methoxy group.

Table 4.3.: *In vitro* Activity Against *P. falciparum* strains W2 (chloroquine resistant) and D10 (chloroquine sensitive)

Compounds	D10 Percentage Survival (10 µg/ml)	D10 IC_{50} (µM)	W2 IC_{50} (µM)
26	107.21%	n/d	>20
27	102.59%	n/d	>20
28	48.62%	n/d	>20
29	56.18%	n/d	>20
30	53.57%	n/d	>20
31	48.75%	n/d	>20
26a	37.67%	1.38	>20
27a	51.68%	n/d	13.74 ± 2.19
28a	33.47%	5.64	10.75 ± 0.44
29a	26.88%	9.02	8.87 ± 2.00
30b	31.01%	0.37	>20
31b	46.37%	n/d	>20
CQ	n/d	0.0598	0.097 ± 0.00092
ART	n/d	n/d	0.019 ± 0.0023

n/d = not determined

Relative to their free ligands (**27** - **29**), the metal complexes **27a** – **29a** showed superior antiplasmodial activity against the W2 strain. Whereas the ligands showed no activity at the highest concentration tested (20 µM), the corresponding metal complexes (**27a** – **29a**) showed activities in the range of 8 – 13 µM. Ligands **30** and **31** and their corresponding complexes (**30b** and **31b**) showed activities greater than IC_{50} = 20 µM.

Overall, complex **29a** showed similar activities against W2 and D10, while none of the free ligands showed appreciable activity against either strain. It is evident that chelation of the ligand to palladium enhances antimalarial activity. This may be through increasing the lipophilic character of the overall complex, and thus favouring permeation through the cell membrane. The differences in antiplasmodial activities observed for the complexes suggest that, 1) the aromatic ring of the coordinated thiosemicarbazone ligand may be involved in the mechanism of inhibition and 2) the effect of the aryl substituent is not electronic

but may be attributed to steric effects. Further studies of the complexes found active as well as their free ligands need to be undertaken in order to ascertain the actual inhibitory effects of these compounds. Additionally, screening of these compounds against mammalian cells is required in order

4.2. Catalytic Studies

The Suzuki-Miyaura cross coupling reaction is considered to be one of the most efficient methods for the construction of carbon-carbon bonds.[35] It involves reaction of an organo-boronic acid with an aryl or alkenyl halide and was first reported by Miyaura and Suzuki in 1979,[36,37] with the 'classic' reaction of phenyl boronic acids and aryl halides being reported in 1981.[38]

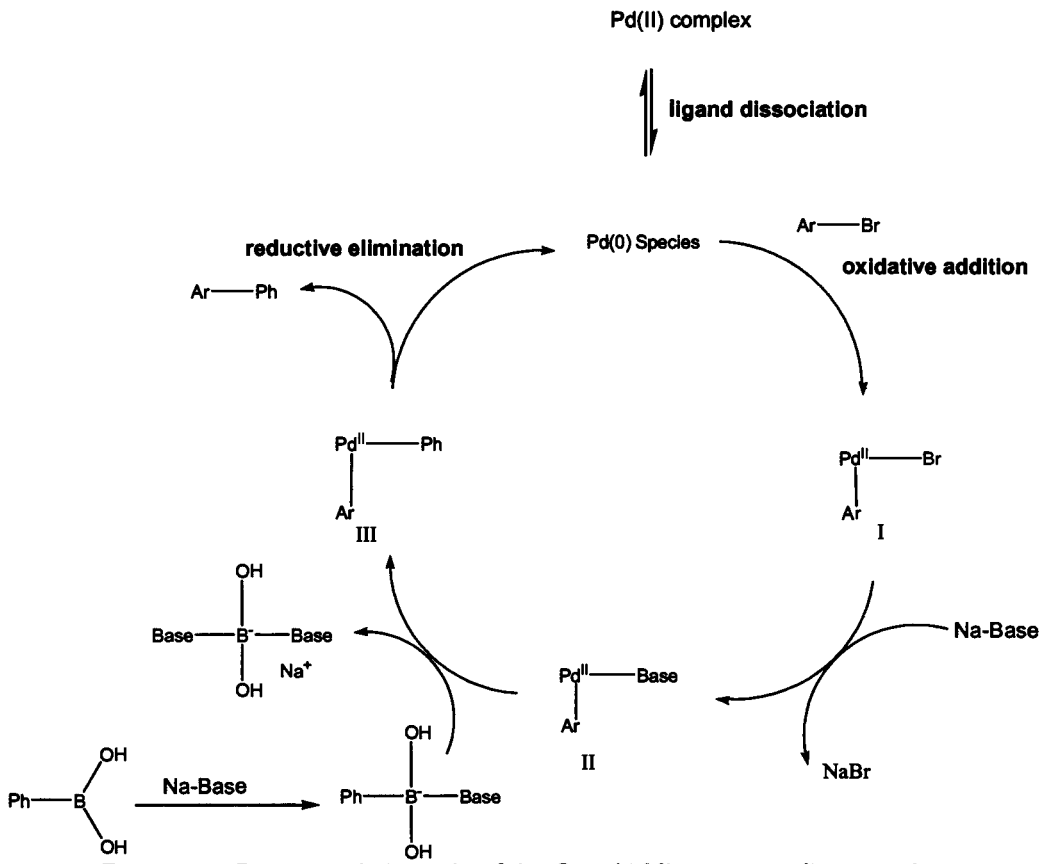
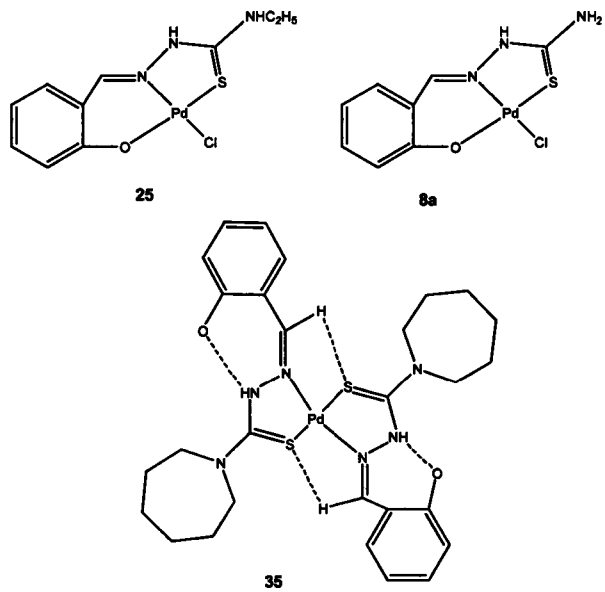


Figure 4.5. Basic catalytic cycle of the Suzuki-Miyaura coupling reaction

Figure 4.5. depicts the generally accepted catalytic cycle of this reaction.[35,39] The palladium(II) complex is reduced *in situ* to the active Pd(0) species, usually by use of

a base, to a zerovalent species which can undergo oxidative addition of the aryl halide to intermediate **I**. Reaction with a base followed by transmetalation of a nucleophilic carbon from boron to the intermediate (**II**) gives **III** which undergoes rapid reductive elimination of the cross-coupled product and the regeneration of the Pd(0) catalyst.[39]

The application of palladium(II) thiosemicarbazone complexes as catalyst precursors for cross-coupling reactions is little known. One of the earliest reports was only published in 2004 describing their use in the Heck coupling reaction.[40] Three salicylaldehyde derived thiosemicarbazone palladium(II) complexes (Figure 4.6.) have been applied to the Suzuki-Miyuara coupling reaction and were shown to catalyze carbon-carbon bond formation in the presence of water.[41,42]

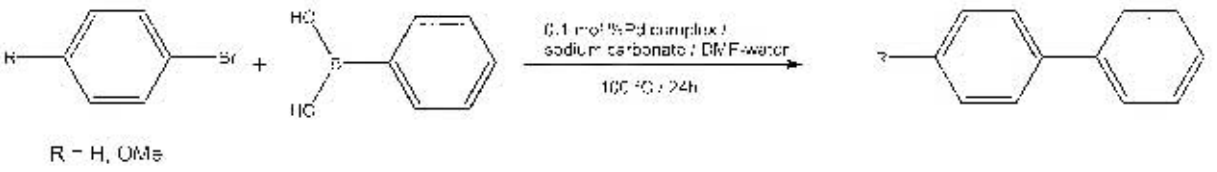


25 8a 35

Figure 4.6.: Structures of Pd(II) complexes previously used for the Suzuki-Miyaura cross-coupling reaction

To test the versatility of the tridentate thiosemicarbazone Pd(II) complexes prepared, complexes **26a – 29a**, **30b** and **31b** were evaluated for preliminary activity as catalyst precursors for the Suzuki-Miyaura coupling reaction (Scheme 4.1.).[41] A 0.1 mol % of each complex was applied to the coupling of either bromobenzene or 4-

bromo-anisole with phenylboronic acid at 100 °C over 24 hours in a DMF-water mixture, using Na_2CO_3 as base. Bromobenzene is a standard substrate used in catalytic studies of aryl-aryl coupling and 4-bromoanisole was chosen since the electron-donating methoxy substituent strengthens the C-Br bond, making oxidative insertion of palladium into the bond difficult.



R = H, OMe

Scheme 4.1.

The products of the reactions were detected using gas chromatography (GC) with decane as internal standard. Figure 4.7. shows the percent conversion of aryl halide substrate and Figure 4.8 shows the TON (turnover number) of each catalyst calculated from GC.

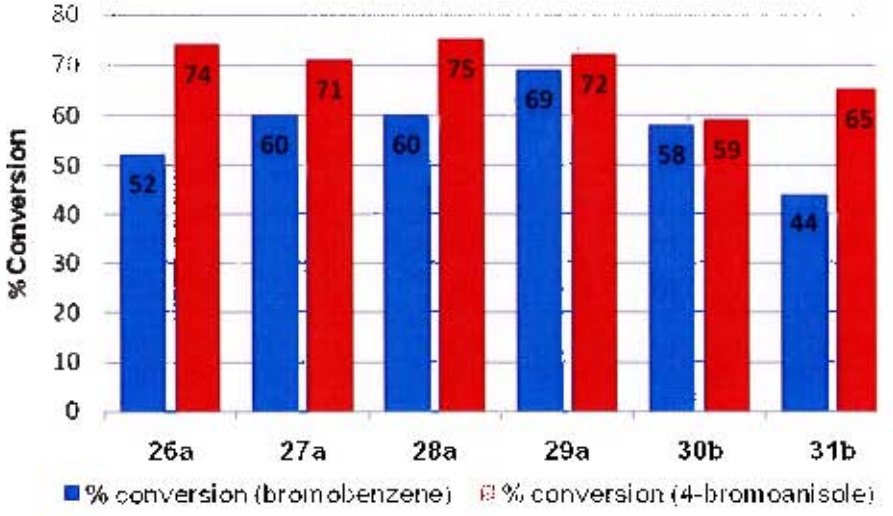


Figure 4.7. % conversions for complexes **26a** **29a, 30b, 31b**

In all of the reactions, the deposition of palladium black was not observed, indicating that the catalysts are stable in air, as well as in the presence of water, at 100°C. All of the complexes showed higher percent conversions for the 4-bromo-anisole

substrate. For complexes **26a** – **29a**, the percent conversions observed for the 4-bromo-anisole substrate were similar to each other, ranging between 71 and 75 %. This is surprising since substrates containing an electron-donating group are known to generally show lower conversions to coupled products. Complex **26a** exhibited a conversion of 52 % for the bromobenzene substrate while complex **29a** showed the highest conversion to product for this substrate.

Complexes **30b** and **31b** were less active than complexes **26a** – **29a**. Similar percent conversions were displayed by **30b** for both aryl bromide substrates and complex **31b** shows the lowest conversion of bromo-benzene out of all the complexes. Overall, complex **29a** was the most effective catalyst showing good conversions for either substrate.

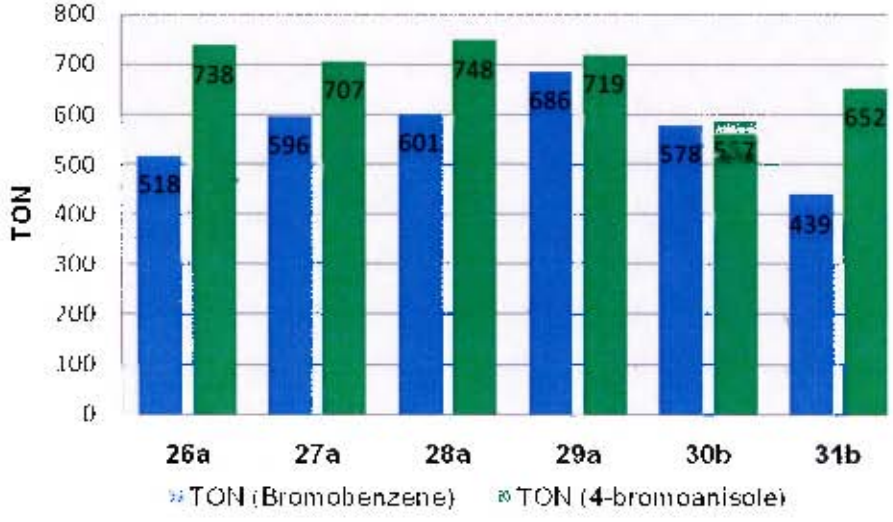


Figure 4.8. TON (turnover number) for complexes **26a** – **29a**, **30b**, **31b**

With regard to actual active palladium species in these reactions, it is unlikely that a Pd(0) species is the active catalyst. All of the complexes used are Pd(II) complexes and dissociation of triphenylphosphine which is a neutral donor leaves Pd in the +2 state. While the generally accepted catalytic cycle for the Suzuki-Miyaura cross coupling reaction is believed to be a Pd(0)/Pd(II) system (Figure 4.5.),[35,39,42] there is another pathway that has been postulated using a Pd(II) complex as active catalyst.[44,45] The basic catalytic cycle possibly utilized by complexes **26a** – **29a**, **30b** and **31b** involving a Pd(II)/Pd(II) catalytic pathway is outlined in Figure 4.8.[45]

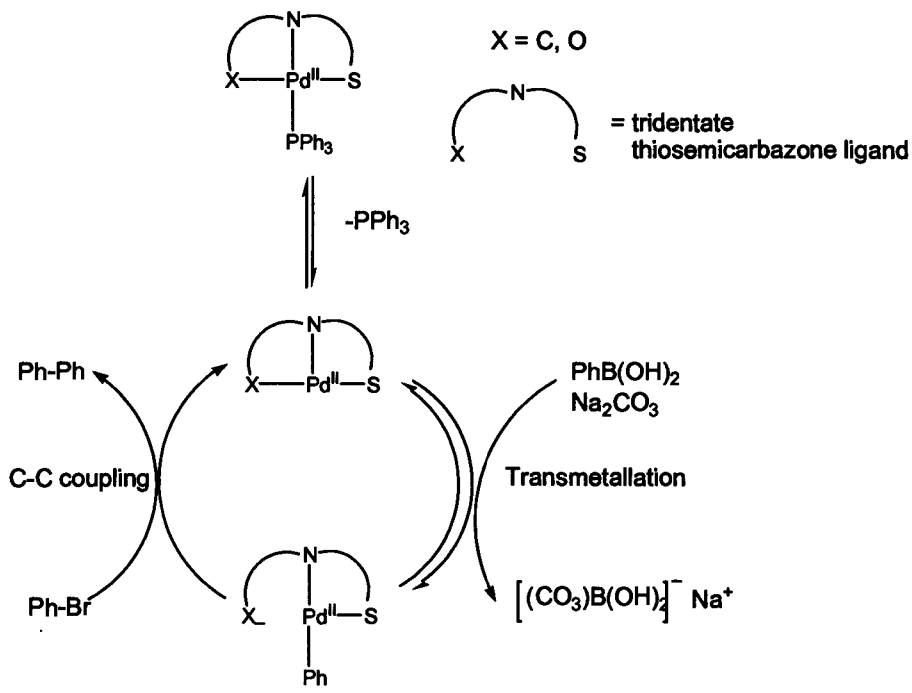


Figure 4.8. Possible catalytic cycle using complexes **26a – 29a**, **30b** and **31b** based on observations discussed in reference 45.

Upon dissociation of triphenylphosphine, rapid transmetallation probably takes place by pre-coordination of the phenyl boronic acid followed by fast metathesis with the aryl bromide to give the coupled product.[45,46] This is only a proposed mechanistic pathway, further studies would need to be carried out in order to ascertain the actual active species. For instance, in order to confidently rule out catalysis by colloidal Pd(0) or molecular Pd(0), the mercury test could be employed. This would determine if the carbon-carbon bond formation is actually catalyzed by a homogenous palladium complex that is in a higher oxidation state.[45] Computational studies as well as NMR studies of the intermediates could also prove effective.

4.3. Conclusion

The preliminary biological evaluation of compounds **26 - 31**, **26a - 29a**, **30b** and **31b** have shown that thiosemicarbazone **27** is an effective cytotoxic agent exhibiting activity against all the cell lines screened. Complexes **28a** and **29a** have also displayed good activity in selected cell lines. PARP cleavage experiments have shown that **27** may induce cell death by apoptosis in the WHCO1, while its mode of inhibition in HeLa cells is uncertain.

Complexes **26a** and **30b** showed superior antiplasmodial activity against the chloroquine sensitive (D10) *P. falciparum* strain. All of the palladium(II) complexes generally exhibited better activity than their corresponding free ligands against either parasite strain.

The presence of a substituent on the aryl ring of the thiosemicarbazone ligand may influence biological activity, however further studies need to be undertaken with regard to structure-activity relationships to ascertain the actual effect.

Complexes **26a** - **29a**, **30b** and **31b** have been shown to successfully catalyze carbon-carbon bond formation between phenyl boronic acid and two aryl bromide substrates. Moderate to good percent conversions were obtained with the complexes exhibiting selectivity towards the 4-bromo-anisole substrate. The results obtained are promising and warrant further investigation into the catalytic activity of these complexes as carbon-carbon cross-coupling catalyst or catalyst precursors.

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Chapter 5: Experimental

5.1. General Experimental Procedures

All complexation reactions were performed under a nitrogen or argon atmosphere using a dual vacuum/nitrogen line and standard Schlenk-line techniques. All reaction solvents were dried by refluxing under an inert atmosphere over the appropriate drying agent and all samples were dried under vacuum. Thiosemicarbazide, 5-chloro-2-hydroxy-benzaldehyde, 3-*tert*-butyl-2-hydroxybenzaldehyde, 2-hydroxy-3-methoxy-benzaldehyde, 2-hydroxy-benzaldehyde, 3,4-dichloro-benzaldehyde, 3,4-dichloro-acetophenone, 3,4-dichloro-propiophenone, 3,5-bis(trifluoromethyl)-benzaldehyde and 3,5-bis(trifluoromethyl)-acetophenone were purchased from Aldrich and used without further purification. Palladium(II) chloride was kindly donated by Johnson-Matthey Inc. Potassium tetrachloropalladate,[1] dichloro(1,5-cyclooctadiene)palladium(II) [2] and dichloro-bis(triphenylphosphine)palladium(II) [3] were prepared according to literature methods.

Nuclear Magnetic Resonance (NMR) spectra were recorded on a Varian Unity XR400 MHz (^{1}H at 399.95 MHz, ^{13}C at 100.58 MHz, ^{31}P at 161.90 MHz, ^{19}F at 376.9 MHz) or Varian Mercury XR300 (^{1}H at 300.08 MHz, ^{13}C at 75.46 MHz, ^{31}P at 121.47 MHz) MHz spectrometer at ambient temperature. Chemical shifts for ^{1}H, ^{19}F{^{1}H} and ^{13}C{^{1}H} NMR shifts are reported using tetramethylsilane (TMS) as the internal standard and ^{31}P{^{1}H} spectra were measured relative to H_3PO_4 as the external standard.

Infrared absorptions (IR) were measured on a Perkin-Elmer Spectrum One FT-IR Spectrometer as KBr pellets. Microanalyses for C, H, N and S were carried out using a Thermo Flash 1112 Series CHNS-O Analyser and melting points were determined using a Kofler hot stage microscope (Reichert Thermovar). Mass Spectrometry determinations were carried out on all new compounds using electron spray

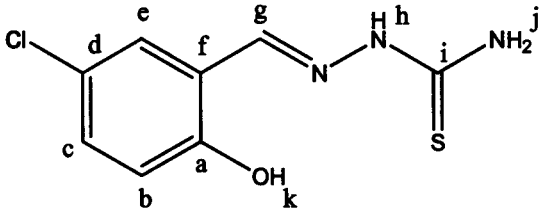
ionisation on a Waters API Quattro Micro instrument in the positive or negative mode.

5.2. Preparation of Thiosemicarbazone Ligands

5.2.1. General Preparation of Thiosemicarbazones

A solution of the appropriate aldehyde or ketone in ethanol (10 cm^3) was added dropwise to a stirred suspension of thiosemicarbazide in ethanol (20 cm^3). Acetic acid (1cm^3) was added to the reactions where a ketone is used. The reaction solution was refluxed, either for 6 hours in the case of the aldehyde thiosemicarbazones or 18-20 hours for the ketone thiosemicarbazones. Upon cooling to room temperature, the product precipitated out of solution and is collected by filtration, washed with ethanol and diethyl ether and dried under vacuum.

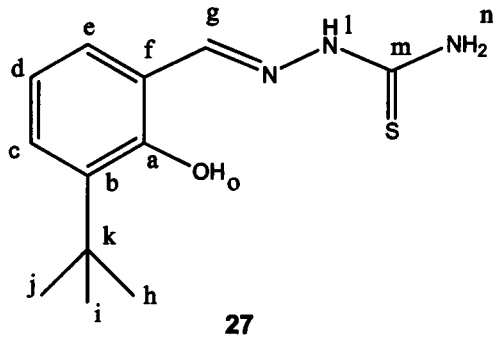
*5.2.1.1. 5-Chloro-2-hydroxybenzaldehyde thiosemicarbazone (**26**)* [4]



26

Thiosemicarbazide (0.168 g, 1.850 mmol) was reacted with 5-chloro-2-hydroxybenzaldehyde (0.203 g, 1.300 mmol). The product (**26**) was isolated as a crystalline white solid (0.131 g, 44%). M.p.: 292-295 °C (lit. m.p.: 290 °C). ^{1}H NMR (300.08 MHz, DMSO): δ (ppm) = 11.33 (br s, 1H, H_i), 10.08 (br s, 1H, H_k) 8.32 (s, 1H, H_g), 8.01 (br s, 3H, H_e, H_j), 7.20 (d, $^3J(H_bH_c)$ = 8.73 Hz, 1H, H_b), 6.87 (d, $^3J(H_cH_b)$ = 8.73 Hz, 1H, H_c). ^{13}C NMR (75.46 MHz, DMSO): δ (ppm) = 178.0 (C_i), 155.0 (C_a), 137.6 (C_g), 130.2 (C_e), 125.4(C_c), 123.3 (C_d), 122.2 (C_b), 117.6 (C_f). IR (KBr, cm^{-1}) υ = 3406 (m, O-H), 3235 (m, N-H), 3166 (s, N-H), 3044 (w, N-H), 1612 (m, C=N), 1542 (s, C=C aromatics), 1099 (m, C=S).

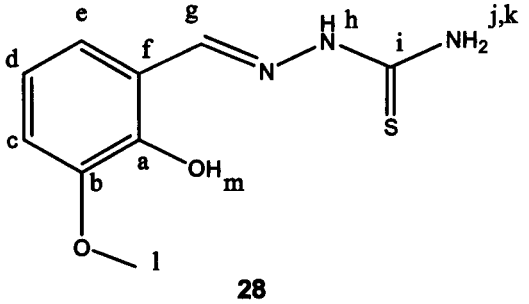
5.2.1.2. 3-tert.-Butyl-2-hydroxybenzaldehyde thiosemicarbazone (27)



27

Thiosemicarbazide (0.271 g, 2.977 mmol) was reacted with 3-*tert.*-butyl-2-hydroxybenzaldehyde (0.520 g, 2.920 mmol). The product (**27**) was isolated as a white solid (0.397 g, 54%). M.p.: 254-257 °C. ^{1}H NMR (300.08 MHz, DMSO): δ (ppm) = 11.29 (s, 1H, H_l), 10.03 (br s, 1H, H_o), 8.28 (s, 1H, H_g), 7.99 (br s, 2H, H_n), 7.25(m, 2H, H_c, H_e), 6.86 (t, $^{3}J(H_cH_dH_e)$ = 7.69 Hz, 1H, H_d), 1.40 (s, 9H, H_h, H_i, H_j). ^{13}C NMR (75.46 MHz, DMSO): δ (ppm) = 177.8 (C_m), 155.3 (C_a), 147.0 (C_g), 136.6 (C_b), 129.3 (C_e), 128.2 (C_c), 119.1 (C_f), 118.4 (C_d), 34.3 (C_k), 29.2 (C_h, C_i, C_j) . IR (KBr, cm^{-1}) υ = 3419 (s, O-H), 3248 (s, N-H), 3164 (s, N-H), 3038 (s, N-H), 1614 (s, C=N), 1537 (s, C=C aromatics), 1148 (m, C=S). Elemental Analysis for $C_{12}H_{17}N_3OS$: found C 56.92 H 6.73 N 16.71 S 12.63 %; calculated C 57.34 H 6.81 N 16.72 S 12.76 %. ESI-MS: *m/z* 252.11 $[M + H]^+$.

5.2.1.3. 2-Hydroxy-3-methoxybenzaldehyde thiosemicarbazone (28) [5]

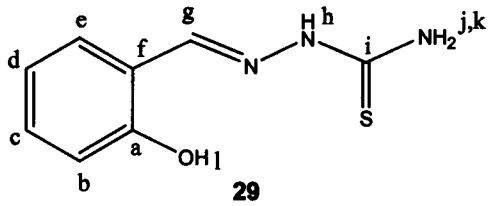


28

Thiosemicarbazide (0.352 g, 3.847 mmol) was reacted with 2-hydroxy-3-methoxybenzaldehyde (0.585 g, 3.847 mmol). The product (**28**) was isolated as a white solid (0.814 g, 94%). M.p.: 216-217 °C (lit. mp.: 220-222 °C). ^{1}H NMR (400 399.95 MHz, DMSO): δ (ppm) = 11.34 (s, 1H, H_h), 9.12 (br s, 1H, H_m), 8.39 (s, 1H, H_g), 8.04 (s, 1H, H_j), 7.82(s, 1H, H_k), 7.50 (d, *J* = 7.80 Hz, 1H, H_e), 6.94 (dd, $^{4}J(H_cH_e)$

= 1.34, $^3J(H_cH_d)$ = 7.98 Hz, 1H, H_c), 6.75 (t, $^3J(H_cH_dH_e)$ = 7.97 Hz, 1H, H_d), 3.79 (s, 3H, H_l). ^{13}C NMR (75.46 MHz, DMSO): δ (ppm) = 177.8 (C_i), 147.8 (C_a), 145.9 (C_b), 139.8 (C_g), 120.6 (C_e), 118.9 (C_c), 118.2 (C_d), 112.9 (C_f), 55.8 (C_l). IR (KBr, cm^{-1}) υ = 3461 (s, O-H), 3344 (s, N-H), 3167 (s, N-H), 3033 (m, N-H), 1598 (s, C=N), 1538 (s, C=C aromatics), 1060 (m, C=S).

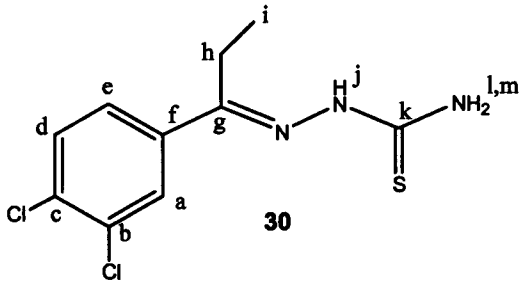
5.2.1.4. 2-Hydroxybenzaldehyde thiosemicarbazone (29) [6]



29

Thiosemicarbazide (0.396 g, 4.346 mmol) was reacted with 2-hydroxybenzaldehyde (0.504 g, 4.129 mmol). The product (**29**) was isolated as a pale yellow solid (0.610 g, 76%). M.p.: 292-295°C (lit. m.p.: 210°C). ^1H NMR (399.95 MHz, DMSO): δ (ppm) = 11.31 (s, 1H, H_h), 9.83 (br s, 1H, H_l), 8.35 (s, 1H, H_g), 8.04 (s, 1H, H_j), 7.85-7.88 (m, 2H, H_e, H_k), 7.17-7.19 (m, 1H, H_c), 6.78-6.86 (m, 2H, H_b, H_d). ^{13}C NMR (75.46 MHz, DMSO): δ (ppm) = 177.8 (C_i), 156.3 (C_a), 140.0 (C_g), 130.9 (C_c), 126.8 (C_e), 120.1 (C_d), 119.1 (C_b), 116.0 (C_f). IR (KBr, cm^{-1}) υ = 3444 (m, O-H), 3319 (s, N-H), 3173 (s, N-H), 3029 (m,br, N-H), 1614 (s, C=N), 1538 (s, C=C aromatics), 1111 (m, C=S).

5.2.1.5. 3,4-Dichloropropiophenone thiosemicarbazone (30) [7]

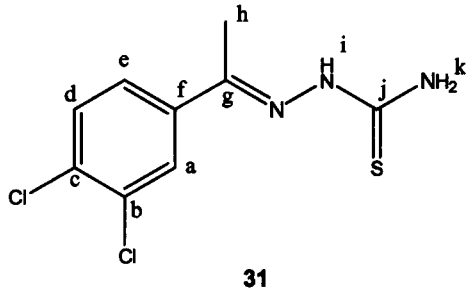


30

Thiosemicarbazide (0.227 g, 2.491 mmol) was reacted with 3,4-dichloropropiophenone (0.500 g, 2.464 mmol). The product (**30**) was isolated as a fluffy white solid (0.184 mmol, 27%). M.p.: 188-190 °C (lit. mp.: 185.6-186.4 °C). ^1H NMR (399.95 MHz, DMSO): δ (ppm) = 10.36 (s, 1H, H_j), 8.31 (s, 1H, H_l), 8.23 (s, 1H, H_a), 8.13 (s, 1H, H_m), 7.86 (dd, $^4J(H_eH_a)$ = 2.80, $^3J(H_eH_d)$ = 8.55 Hz, 1H, H_e), 7.61 (d,

$^3J(H_dH_e)$ = 8.54 Hz, 1H, H_d), 2.86 (q, J = 7.55, 7.55 Hz, 2H, H_h), 0.98 (t, J = 9.41Hz, 3H, H_i). ^{13}C NMR (75.46 MHz, DMSO): δ (ppm) = 179.2 (C_k), 149.3 (C_g), 136.9 (C_f), 131.5 (C_c), 131.4 (C_b), 130.2 (C_a), 128.2 (C_d), 126.7 (C_e), 18.9 (C_h), 10.5 (C_i). IR (KBr, cm^{-1}) υ = 3389 (m, N-H), 3254 (m, N-H), 3174 (m, N-H), 1595 (m, C=N), 1512 (s, C=C aromatics), 1054 (s, C=S).

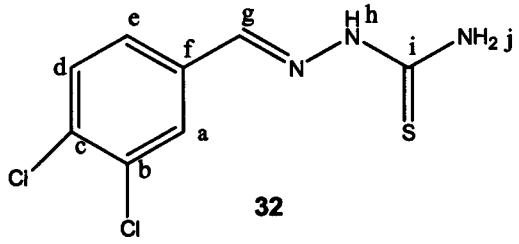
*5.2.1.6. 3,4-Dichloroacetophenone thiosemicarbazone (**31**)* [7]



31

Thiosemicarbazide (0.546 g, 5.987 mmol) was reacted with 3,4-dichloroacetophenone (1.002 g, 5.303 mmol). The product (**31**) was isolated as a fluffy white solid (0.485 g, 35%). M.p.: 191-192 °C (lit. mp.: 196 – 198 °C). ^1H NMR (300.08 MHz, DMSO): δ (ppm) = 10.20 (br s, 1H, H_i), 8.26 (s, 1H, H_a), 8.13 (br s, 2H, H_k), 7.90 (d, $^3J(H_dH_e)$ = 8.52 Hz, 1H, H_d), 7.66 (d, $^3J(H_eH_d)$ = 8.52 Hz, 1H, H_e), 2.33 (s, 3H, H_h). ^{13}C NMR (75.46 MHz, DMSO): δ (ppm) = 179.1 (C_j), 145.2 (C_g), 138.2 (C_f), 131.5 (C_c), 131.2 (C_b), 130.0 (C_d), 128.0 (C_a), 126.5 (C_e), 13.6 (C_h). IR (KBr, cm^{-1}) υ = 3421 (s, N-H), 3181 (m, N-H), 3141 (m, N-H), 1594 (s, C=N), 1091 (s, C=S).

*5.2.1.7. 3,4-Dichloro-benzaldehyde thiosemicarbazone (**32**)* [7]

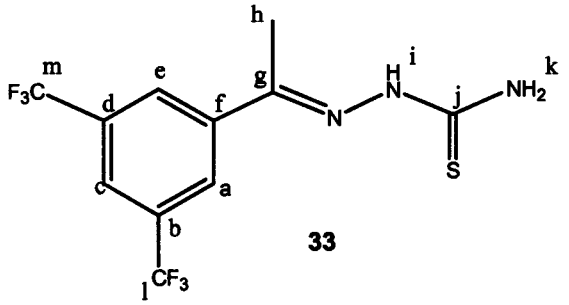


32

Thiosemicarbazide (0.131 g, 1.436 mmol) was reacted with 3,4-dichlorobenzaldehyde (0.225 g, 1.287 mmol). The product (**32**) was isolated as a fluffy white solid (0.135 g, 42%). M.p.: 195-198°C (lit. mp.: 202-204 °C[8]). ^1H NMR (399.95 MHz, DMSO): δ (ppm) = 11.51 (s, 1H, H_h) 8.21 (br s, 3H, H_g, H_j), 7.98 (s, 1H, H_a), 7.71 (d, $^3J(H_dH_e)$ = 8.37 Hz, 1H, H_d), 7.63 (d, $^3J(H_eH_d)$ = 8.33 Hz, 1H, H_e).

^{13}C NMR (75.46 MHz, DMSO): δ (ppm) = 178.3 (C_i), 139.4 (C_g), 134.9 (C_c), 131.7 (C_b), 131.6 (C_f), 130.5 (C_d), 128.0 (C_a), 127.4 (C_e). IR (KBr, cm^{-1}) υ = 3397 (m, N-H), 3256 (m, N-H), 3156 (s, N-H), 1597 (s, C=N), 1540 (s, C=C aromatics), 1103 (m, C=S).

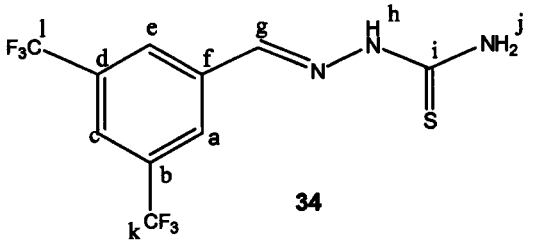
*5.2.1.8. 3,5-Bis(trifluoromethyl)acetophenone thiosemicarbazone (**33**)* [7]



33

Thiosemicarbazide (0.074 g, 0.8185 mmol) was reacted with 3,5-bis(trifluoromethyl)acetophenone (0.213 g, 0.833 mmol). The product (**33**) was isolated as a fluffy white solid (0.111 g, 41%). M.p.: decomposition without melting 240-241 °C (lit. m.p.: decomposition without melting 238 °C). ^1H NMR (399.95 MHz, DMSO): δ (ppm) = 10.33 (s, 1H, H_i), 8.52 (s, 2H, H_a, H_e), 8.33 (s, 2H, H_k), 8.05 (s, 1H, H_c), 2.34 (s,3H, H_h). ^{13}C NMR (100.58 MHz, DMSO): δ (ppm) = 180.1 (C_j), 145.9 (C_g), 141.2 (C_f), 131.3 (C_b, C_d), 127.9 (C_a, C_e), 125.4 (C_c), 122.7 (C_l, C_m), 14.9 (C_h). ^{19}F NMR (376.9 MHz, $CDCl_3$): δ = -61.59 ($C\underline{F}_3$). IR (KBr, cm^{-1}) υ = 3436 (m, N-H), 3257 (s, N-H), 3163 (s, N-H), 1602 (s, C=N), 1507 (m, C=C aromatics), 1118 (m, C=S).

*5.2.1.9. 3,5-Bis(trifluoromethyl)benzaldehyde thiosemicarbazone (**34**)* [7]



34

Thiosemicarbazide (0.189 g, 2.077 mmol) was reacted with 3,5-bis(trifluoromethyl) benzaldehyde (0.500 g, 2.065 mmol). The product (**34**) was isolated as a fluffy white solid (0.176 g, 27%). M.p.: 225-227 °C (lit. m.p.: 229.8-230.3 °C). ^1H NMR (399.95

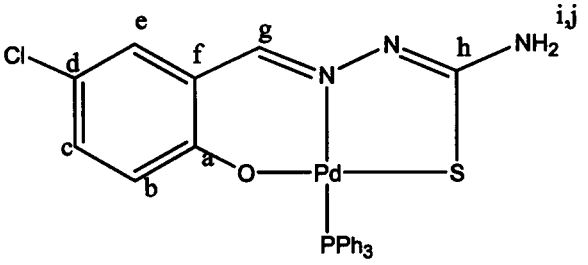
MHz, DMSO): δ (ppm) = 11.68 (s, 1H, H_h), 8.52 (s, 2H, H_a, H_e), 8.43 (s, 1H, H_j), 8.34 (s, 1H, H_j), 8.16 (s,1H, H_g), 8.03 (s, 1H, H_c). ^{13}C NMR (100.58 MHz, DMSO): δ (ppm) = 179.4 (C_i), 139.7 (C_g), 138.0 (C_f), 132.0 (C_b, C_d), 128.5 (C_a, C_e), 125.4 (C_c), 123.0 (C_k, C_l). ^{19}F NMR (376.9 MHz, $CDCl_3$): δ = -61.74 ($C\underline{F}_3$). IR (KBr, cm^{-1}) υ = 3451 (s, N-H), 3303 (s, N-H), 3174 (s, N-H), 1601 (s, C=N), 1535 (s, C=C aromatics), 1152 (s, C=S).

5.3. Preparation of Palladium Complexes

5.3.1. General Preparation of [Pd(Salicylaldiminato thiosemicarbazone)(PPh_3)] Complexes

The appropriate thiosemicarbazone ligand (1 mol equiv.) was added to dry ethanol (40 cm^3) and the solution heated to 60°C with stirring. Triethylamine (2.1 mol equiv.) was added followed by $Pd(PPh_3)_2Cl_2$ (1 mol equiv, complexes **26a** – **29a**) or Pd(4-pic)$_2Cl_2$ (1 mol equiv, complexes **26b** – **29b**) and the resulting mixture was refluxed for five hours unless otherwise stated. Upon cooling to room temperature, the precipitated product was isolated via filtration, washed with ethanol (10 cm^3) and diethyl ether (10 cm^3). Complexes **26a-29a** were purified by recrystallization from a mixture of DCM and hexane and complexes **26b** – **29b** were recrystallized from chloroform.

*5.3.1.1. [Pd(5-Chloro-2-hydroxybenzaldehyde thiosemicarbazone)(PPh_3)] (**26a**)*

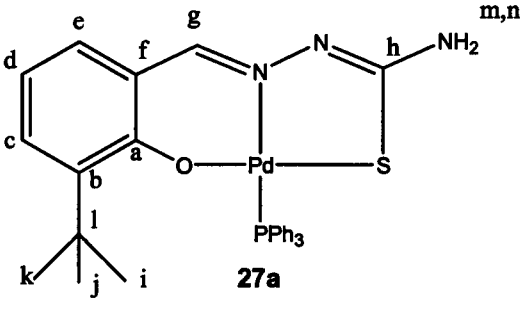


26a

Dichlorobis(triphenylphosphine)palladium(II) (0.513 g, 0.731 mmol) was reacted with 5-chloro-2-hydroxybenzaldehyde thiosemicarbazone (0.162 g, 0.706 mmol). The reaction was refluxed for 2 hours and the crude product precipitated out of solution. Recrystallization from DCM-Hexane yielded the product (**26a**) as a crystalline yellow

solid (0.262 g, 63% yield). M.p.: 221-223 °C. ^{1}H NMR (300.08 MHz, $CDCl_3$): δ(ppm) = 8.13 (d, $^{4}J_{PH}$ = 13.62 Hz, 1H, H_g), 7.66-7.75 (m, 6H, PPh_3), 7.26-7.55 (m, 9H, PPh_3), 7.23 (d, $^{4}J(H_eH_c)$ = 2.75, 1H, H_e), 7.11 (d, $^{3}J(H_cH_b)$ = 9.02 Hz, 1H, H_c), 6.59 (d, $^{3}J(H_bH_c)$ = 9.02 Hz, 1H, H_b), 4.74 (s, 2H, H_i, H_j). ^{13}C NMR (75.46 MHz, $CDCl_3$): δ (ppm) = 171.5 (C_g), 161.2 (C_h), 149.6 (C_a), 128.3-134.7 (PPh_3, C_c, C_d and C_e), 122.2 (C_f), 118.7 (C_b). ^{31}P NMR (121.47 MHz, $CDCl_3$): δ (ppm) = 25.23 (PPh_3). IR (KBr, cm^{-1}) υ = 3493 (N-H), 3385 (m, N-H), 3054 (w, C-N), 1605 (s, C=N), 1586 (m, C=N), 1529 (s, C=C aromatics). Elemental Analysis for $C_{26}H_{21}ClN_3OPPdS$: found C 51.96 H 3.55 N 5.83 S 4.76 %; calculated C 52.36 H 3.55 N 7.05 S 5.38 %. ESI-MS: *m/z* 597 $[M + H]^+$, 100%.

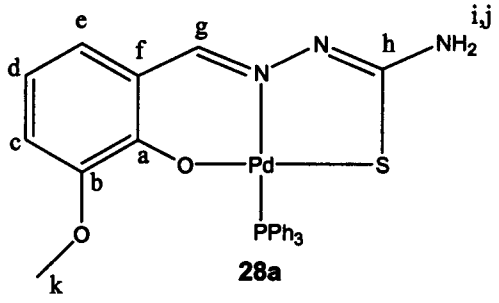
*5.3.1.2. [Pd(3-tert.-Butyl-2-hydroxybenzaldehyde thiosemicarbazone)(PPh_3)] (**27a**)*



27a

Dichlorobis(triphenylphosphine)palladium(II) (0.530g, 0.755 mmol) was reacted with 3-*tert.*-butyl-2-hydroxybenzaldehyde thiosemicarbazone (0.215 g, 0.837 mmol). The reaction solution was refluxed for 4 hours and the crude product precipitated out of solution. Recrystallization from DCM-Hexane yields the product (**27a**) as a crystalline orange solid (0.136 g, 29%). M.p.: 234-236°C. ^{1}H NMR (399.95 MHz, $CDCl_3$): δ (ppm) = 8.26 (d, $^{4}J_{PH}$ = 14.14 Hz, 1H, H_g), 7.70-7.77 (m, 6H, PPh_3), 7.38-7.48 (m, 9H, PPh_3), 7.20-7.37 (m, 2H, H_c, H_e), 6.57 (t, $^{3}J(H_cH_dH_e)$ = 8.82 Hz, 1H, H_d), 4.59 (s, 2H, H_m, H_n), 0.744 (s, 9H, H_i, H_j, H_k). ^{13}C NMR (100.58 MHz, $CDCl_3$): δ (ppm) = 170.0 (C_g), 162.2 (C_h), 152.3 (C_a), 140.3 (C_b), 128.0-135.1 (PPh_3, C_e, C_c), 118.0 (C_d), 114.1 (C_f), 34.79 (C_l), 29.44 ($C_{i,j,k}$). ^{31}P NMR (161.90 MHz, $CDCl_3$): δ (ppm) = 24.16 (PPh_3). IR (KBr, cm^{-1}) υ = 3464 (m, N-H), 3391 (m, N-H), 1634 (m, C=N), 1610 (s, C=N), 1593 (s, C=C aromatics). Elemental Analysis for $C_{30}H_{30}N_3OPPdS$: found C 57.73 H 4.91 N 6.39 S 4.69 %; calculated C 58.30 H 4.89 N 6.80 S 5.19 %. ESI-MS: *m/z* 617 $[M - H]^+$, 100%.

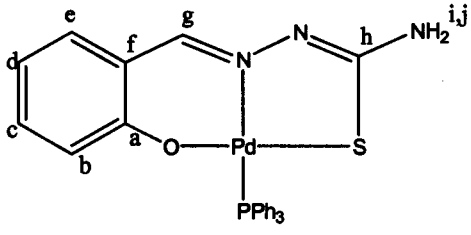
*5.3.1.3. [Pd(2-Hydroxy-3-methoxybenzaldehyde thiosemicarbazone)(PPh_3)] (**28a**)*



28a

Dichlorobis(triphenylphosphine)palladium(II) (0.516 g, 0.735 mmol) was reacted with 2-hydroxy-3-methoxybenzaldehyde thiosemicarbazone (0.164 g, 0.728 mmol). The reaction was refluxed for 6 hours and the crude product precipitated out of solution. Recrystallization from DCM-Hexane yields the product (**28a**) as a dark orange crystalline solid (0.344 g, 80% yield). M.p.: 241-243 °C. ^{1}H NMR (300.08 MHz, $CDCl_3$): δ (ppm)= 8.24 (d, $^4J_{PH}$ = 13.87 Hz, 1H, H_g), 7.49-7.81 (m, 6H, PPh_3), 7.26-7.49 (m, 9H, PPh_3), 6.96 (d, $^3J(H_eH_d)$ = 8.06 Hz, 1H, H_e), 6.85 (d, $^3J(H_cH_d)$ = 7.57 Hz, 1H, H_c), 6.56 (t, $^3J(H_cH_dH_e)$ = 7.79 Hz, 1H, H_d), 4.67 (s, 2H, H_i, H_j), 3.57 (s, 3H, H_k). ^{13}C NMR (75.46 MHz, $CDCl_3$): δ (ppm) = 170.4 (C_g), 150.9 (C_h and C_b), 128.3-134.8 (PPh_3, C_a), 126.5 (C_e), 117.7 (C_d), 115.5 (C_f), 113.9 (C_c), 56.6 (C_k). ^{31}P NMR (121.47 MHz, $CDCl_3$): δ (ppm) = 19.67 (PPh_3). IR (KBr, cm^{-1}) υ = 3442 (m, N-H), 3308 (w, N-H), 1642 (m, C=N), 1592 (s, C=N), 1526 (s, C=C aromatics). Elemental Analysis for $C_{27}H_{24}N_3O_2PPdS$: found C 53.67 H 4.32 N 6.10 S 5.17 %; calculated C 54.78 H 4.09 N 7.10 S 5.42%. ESI-MS: *m/z* 591 $[M - H]^+$, 100%.

*5.3.1.4. [Pd(2-hydroxybenzaldehyde thiosemicarbazone)(PPh_3)] (**29a**)* [9]

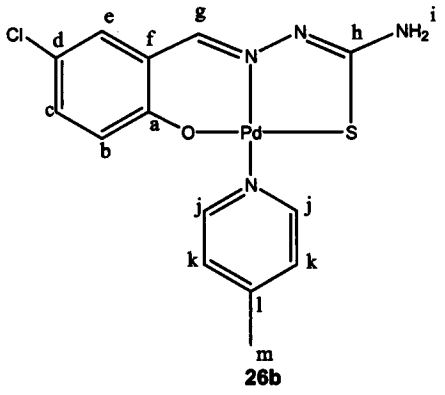


29a

Dichlorobis(triphenylphosphine)palladium(II) (0.514 g, 0.733 mmol) was reacted with 2-hydroxybenzaldehyde thiosemicarbazone (0.146 g, 0.746 mmol). The reaction solvent was concentrated under reduced pressure and the crude product precipitated out of solution. Recrystallization from DCM-Hexane yielded the product (**29a**) as a crystalline orange solid (0.149 g, 36%). M.p.: 228-231°C (lit. mp.: 230-232

°C [10]). ^{1}H NMR (399.95 MHz, $CDCl_3$): δ (ppm) = 8.27 (d, $^4J_{PH}$ = 13.50 Hz, 1H, H_g), 7.70-7.75 (m, 6H, PPh_3), 7.50-7.53 (m, 3H, PPh_3), 7.43-7.48 (m, 7H, H_d and PPh_3), 7.28-7.43 (m, 3H, H_b, H_c, H_e), 4.69 (s, 2H, H_i, H_j). ^{13}C NMR (75.46 MHz, $CDCl_3$): δ (ppm) = 171.0 (C_g), 162.7 (C_h), 150.7 (C_a), 128.3-134.7 (PPh_3, C_e, C_c), 120.7 (C_d), 117.6 (C_f), 114.8 (C_b). ^{31}P NMR (121.47 MHz, $CDCl_3$)): δ (ppm) = 24.97 (PPh_3). IR (KBr, cm^{-1}) υ = 3300 (w, N-H), 3132 (w, N-H), 1638 (m, C=N), 1599 (s, C=N), 1518 (s, C=C aromatics).

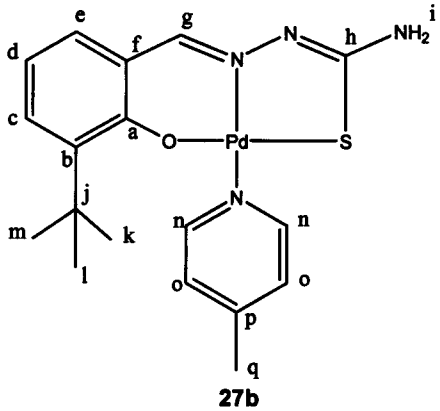
*5.3.1.5. [Pd(5-Chloro-2-hydroxybenzaldehyde thiosemicarbazone)(4-pic)] (**26b**)*



26b

Dichlorobis(4-picoline)palladium(II) (0.394g, 1.089 mmol) was reacted with 5-chloro-2-hydroxybenzaldehyde thiosemicarbazone (0.246 g, 1.070 mmol). The product (**26b**) precipitated out of solution as a yellow solid (0.137 g, 30 %). M.p.: decomposition without melting 238-240 °C. ^{1}H NMR (300.08 MHz, $CDCl_3$): δ (ppm) = 8.59 (d, $^3J(H_jH_k)$ = 6.60 Hz, 2H, H_j), 7.83 (s, 1H, H_g), 7.16-7.44 (m, 3H, H_e, H_k), 6.94 - 6.96 (m, 2H, H_b, H_c), 4.78 (s, 2H, H_i), 2.44 (s, 3H, H_m). ^{13}C NMR (75.46 MHz, $CDCl_3$): δ (ppm) 172.71 (C_g), 161.09 (C_h), 152.3 (C_a), 151.8 (C_j), 147.9 (C_l), 132.7 (C_d), 132.3 (C_e), 127.5 (C_k), 121.9 (C_c), 120.5 (C_f), 117.9 (C_b), 21.29 (C_m). IR (KBr, cm^{-1}) υ = 3403 (w, N-H), 3273 (w, N-H), 1622 (s, C=N), 1596 (s, C=N), 1518 (s, C=C aromatics). Elemental Analysis for $C_{14}H_{13}N_4ClOPdS$: found C 40.76, H 3.40, N 13.86, S 7.43 %; calculated C 39.36, H 3.07, N 13.11, S 7.51 %. ESI-MS: *m/z* 429 ($[M + 2H]^{2+}$, 100%).

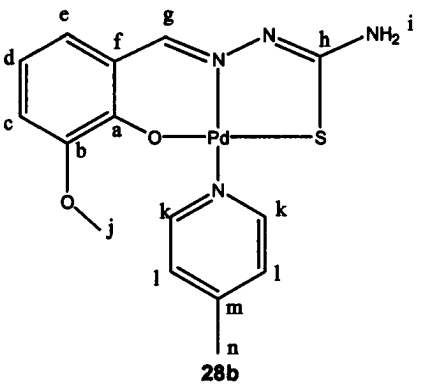
5.3.1.6. [Pd(3-tert.-Butyl-2-hydroxybenzaldehyde thiosemicarbazone)(4-pic)] ***(27b)***



27b

Dichlorobis(4-picoline)palladium(II) (0.501g, 1.385 mmol) was reacted with 3-*tert.*-butyl-2-hydroxybenzaldehyde thiosemicarbazone (0.349 g, 1.387 mmol). The reaction mixture was refluxed for 5 hours and the product (**27b**) precipitated out of solution as a fluffy yellow solid (0.285 g, 46%). M.p.: no melting or decomposition up to 310 °C. ^{1}H NMR (399.95 MHz, $CDCl_3$): δ (ppm) = 8.69 (d, $^{3}J(H_nH_o)$ = 6.50 Hz, 2H, H_n), 7.97 (s, 1H, H_g), 7.32-7.36 (m, 1H, H_e), 7.18-7.25 (m, 3H, H_d, H_o), 6.79-6.42 (m, 1H, H_c), 4.73 (s, 2H, H_i), 2.47 (s, 3H, H_q), 1.33 (s, 9H, H_k, H_l, H_m). ^{13}C NMR (75.46 MHz, $CDCl_3$): δ (ppm) = 169.8 (C_g), 161.8 (C_h), 152.0 (C_a), 151.7 (C_n), 150.8 (C_q), 138.9 (C_b), 132.6 (C_c), 130.3 (C_e), 125.9 (C_o), 118.7 (C_d), 114.3 (C_f), 35.2 (C_j), 29.4 (C_k, C_l, C_m), 21.2 (C_q). IR (KBr, cm^{-1}) υ = 3421 (m, N-H), 3310 (s, N-H), 1621 (br w, C=N), 1609 (s, C=N), 1591 (s, C=N), 1527 (s, C=C aromatics). Elemental Analysis for $C_{18}H_{22}N_4OPdS$: found C 47.81, H 5.03, N 13.01, S 6.96 %; calculated C 48.16, H 4.94, N 12.48, S 7.14 %. ESI-MS: *m/z* 449 ($[M]^+$, 100%).

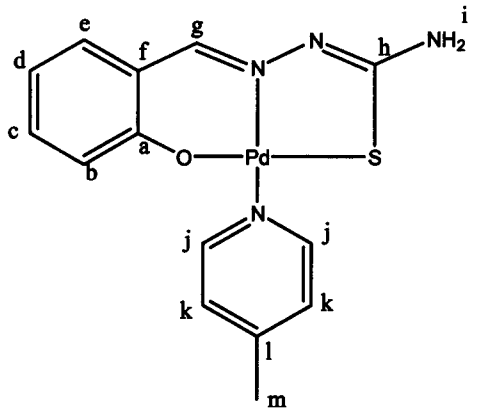
*5.3.1.7. [Pd(2-Hydroxy-3-methoxybenzaldehyde thiosemicarbazone)(4-pic)] (**28b**)*



28b

Dichlorobis(4-picoline)palladium(II) (0.501 g, 1.385 mmol) was reacted with 2-hydroxy-3-methoxybenzaldehyde thiosemicarbazone (0.312 g, 1.385 mmol). The reaction mixture was heated to 50 °C for 2 hours. The reaction was then filtered and the volume of the filtrate reduced before being cooled, the product (**28b**) precipitated as a crystalline orange solid (0.155 g, 27 %). M.p.: 225-227 °C. ^{1}H NMR (300.08 MHz, $CDCl_3$): δ (ppm) = 8.70 (d, $^{3}J(H_kH_l)$ = 6.60 Hz, 2H, H_k), 7.97 (s, 1H, H_g), 7.14-7.31 (m, 1H, H_e), 6.95 -7.02 (m, 3H, H_d, H_l), 6.52-6.68 (m, 1H, H_c), 4.74 (s, 2H, H_i), 3.85 (s, 3H, H_j), 2.43 (s, 3H, H_n). ^{13}C NMR (75.46 MHz, $CDCl_3$): δ (ppm) = 170.5 (C_g), 153.9 (C_h), 152.2 (C_b), 151.8 (C_k), 150.7 (C_m), 149.0 (C_a), 127.4 (C_l), 126.2 (C_e), 125.3 (C_d), 119.1 (C_f), 114.5 (C_c), 56.7 (C_j), 21.2 (C_n). IR (KBr, cm^{-1}) υ = 3428 (br m, N-H), 3351 (br m, N-H), 1621(s, C=N), 1603 (s, C=N), 1525 (s, C=C aromatics). Elemental Analysis for $C_{15}H_{16}N_4O_2PdS$ ⅓$CHCl_3$ ½C_6H_{14}: found C 43.81, H 4.20, N 11.88, S 6.38 %; calculated C 43.54, H 4.65, N 11.88, S 6.34 %. ESI-MS: *m/z* 423 ($[M]^+$, 100%).

*5.3.1.8. [Pd(2-Hydroxybenzaldehyde thiosemicarbazone)(4-pic)] (**29b**)*



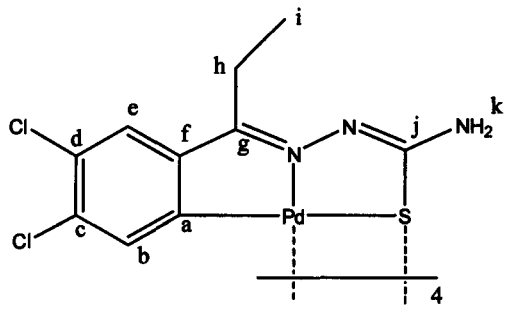
29b

Dichlorobis(4-picoline)palladium(II) (0.502 g, 1.382 mmol) was reacted with 2-hydroxybenzaldehyde thiosemicarbazone (0.271 g, 1.387 mmol). Reaction solvent was concentrated under reduced pressure and the product (**29b**) precipitated out of solution as a crystalline orange solid (0.089 g, 34%). M.p.: decomposition without melting 185-187 °C. ^{1}H NMR (399.95 MHz, $CDCl_3$): δ (ppm) = 8.68 (d, $^3J(H_jH_k)$ = 6.57 Hz, 2H, H_j), 7.98 (s, 1H, H_g), 7.32-7.39 (m, 2H, H_c, H_e), 7.29 (d, $^3J(H_kH_j)$ = 5.96 Hz, 2H, H_k), 7.09 (d, $^3J(H_bH_c)$ = 8.95, 1H, H_b), 6.73 (t, $^3J(H_cH_dH_e)$ = 7.38, 1H, H_d), 4.85 (s, 2H, H_i), 2.49 (s, 3H, H_m). ^{13}C NMR (75.46 MHz, $CDCl_3$): δ (ppm) = 170.0 (C_g), 162.6 (C_h), 151.6 (C_a), 150.97 (C_j), 150.8 (C_l), 133.9 (C_c), 133.6 (C_e), 126.3 (C_k), 119.9 (C_d), 118.4 (C_f), 115.1 (C_b), 21.15 (C_m). IR (KBr, cm^{-1}) υ = 3434 (w, N-H), 3278 (w, N-H), 1625 (s, C=N), 1600 (s, C=N), 1588 (m, C=N), 1527 (s, C=C aromatics).

5.3.2. General Preparation of Tetrameric Thiosemicarbazone Palladium Complexes

Potassium tetrachloropalladate (1 mol. equiv.) was dissolved in deionized water (5 cm^3). Ethanol (40 cm^3) was added and the appropriate thiosemicarbazone ligand (1.1 mol. equiv.) was added to the resulting yellow suspension. The reaction mixture was stirred for 48 hours. The product was collected as a yellow solid via filtration, washed with ethanol (3 x 10 cm^3) and dried under vacuum.

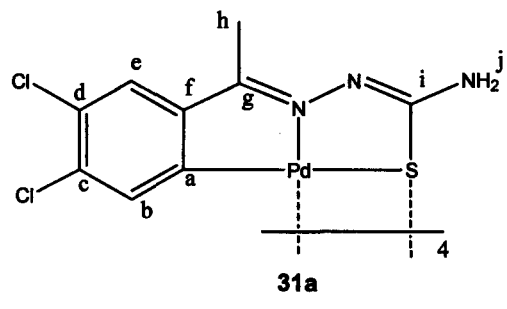
*5.3.2.1. [Pd(3,4-Dichloro-propiophenone thiosemicarbazone)]$_4$ (**30a**)*



30a

Potassium tetrachloropalladate (0.231g, 0.709 mmol) was reacted with 3,4-dichloro-propiophenone thiosemicarbazone (0.201g, 0.773 mmol). The product (**30a**) was isolated as a yellow solid by filtration (0.166 g, 61 %). M.p.: decomposition without melting 309-311 °C. ^{1}H NMR (300.08 MHz, DMSO): δ (ppm) = 7.36 (s, 1H, H_b), 7.11 (s, 2H, H_k), 6.68 (s, 1H, H_e), 2.48 (dt, *J* = 1.88, 3.74 Hz, 2H, H_h), 0.925 (t, *J* = 7.52 Hz, 3H, H_i). ^{13}C NMR (100.58 MHz, DMSO): δ (ppm) = 170.8 (C_j), 168.1 (C_g), 165.5 (C_a), 149.4 (C_f), 134.2 (C_c), 130.3 (C_d), 126.9 (C_e), 126.3 (C_b), 21.1 (C_h), 12.0 (C_i). IR (KBr, cm^{-1}) υ = 3459 (m, N-H), 3376 (m, N-H), 1595 (s, C=N), 1561 (m, C=N), 1525 (s, C=C aromatics). Elemental Analysis for $C_{40}H_{36}Cl_8N_{12}Pd_4S_4$: found C 31.36, H 2.60, N 10.10, S 10.10 %; calculated C 31.56, H 2.38, N 11.04, S 8.43 %. ESI-MS: *m/z* 1522 ($[C_{40}H_{36}Cl_8N_{12}Pd_4S_4]^+$, 20%), 382 ($[C_{10}H_9Cl_2N_3PdS]^+$, 100%).

*5.3.2.2. [Pd(3,4-Dichloro-acetophenone thiosemicarbazone)]$_4$ (**31a**)*



31a

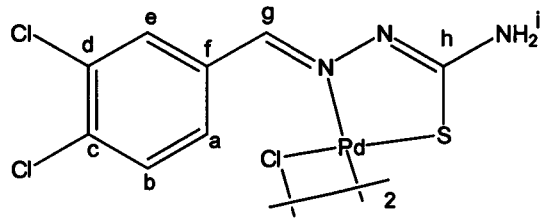
Potassium tetrachloropalladate (0.402 g, 1.230 mmol) was reacted with 3,4-dichloro-acetophenone thiosemicarbazone (0.355 g, 1.356 mmol). The product (**31a**) precipitates out of solution as a yellow solid (0.326 g, 73 %). M.p.: decomposition without melting 307-309 °C. ^{1}H NMR (399.95 MHz, DMSO): δ (ppm) = 7.36 (s, 1H, H_b), 7.17 (s, 2H, H_j), 6.71 (s, 1H, H_e), 1.99 (s, 3H, H_h). ^{13}C NMR (75.46 MHz, DMSO): δ (ppm) = 167.7 (C_i), 166.1 (C_g), 165.1 (C_a), 150.7 (C_f), 133.9 (C_c), 129.9

(C_d), 126.8 (C_e), 126.6 (C_b), 14.3 (C_h). IR (KBr, cm^{-1}) υ = 3442 (m, N-H), 3369 (m, N-H), 1597 (s, C=N), 1560 (m, C=N), 1524 (s, C=C aromatics). Elemental Analysis for $C_{36}H_{28}Cl_8N_{12}Pd_4S_4$: found C 29.14, H 2.43, N 10.87, S 8.56 %; calculated C 29.44, H 1.93, N 11.46, S 8.76 %. ESI-MS: *m/z* 1468 ($[C_{36}H_{28}Cl_8N_{12}Pd_4S_4 + 2H]^+$, 20%), 368 ($[C_9H_7Cl_2N_3PdS + H]^+$, 100%).

5.3.3. General Preparation of Dimeric Thiosemicarbazone Palladium Complexes

The ligand (1 mol equiv.) was added to a stirred suspension of potassium tetrachloropalladate (1.1 mol equiv.) in dry ethanol (15 cm^3) and the resulting reaction mixture was heated to 45 °C for 24 hours. The reaction was then allowed to cool to room temperature and the product isolated via filtration, washed with cold ethanol (2 x 10 cm^3) and dried under vacuum.

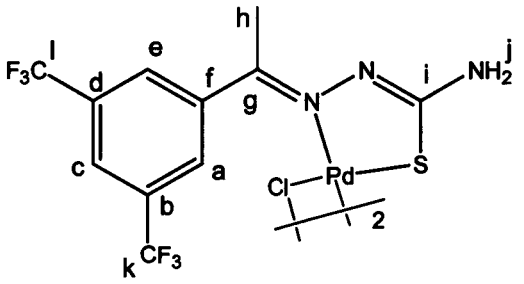
*5.3.3.1. [Pd(3,4-Dichloro-benzaldehyde thiosemicarbazone)Cl]$_2$ (**32a**)*



32a

The ligand, 3,4-dichloro-benzaldehyde thiosemicarbazone (0.109 g, 0.440mmol) was reacted with potassium tetrachloropalladate (0.155 g, 0.475 mmol). The crude product (**32a**) was isolated as an orange solid (0.228 g, 45 % crude yield). M.p.: decomp. without melting 255-256 °C. ^{1}H NMR (300.08 MHz, DMSO): δ (ppm) = 8.52 (s, 1H, H_g), 8.24 (s, 1H, H_e), 7.91 (dd, $^4J(H_aH_e)$ = 1.86, $^3J(H_aH_b)$ = 8.53 Hz, 1H, H_a), 7.63 (d, $^3J(H_bH_a)$ = 8.50 Hz, 1H, H_b), 7.09 (s, 2H, H_i). ^{13}C NMR (75.46 MHz, DMSO): δ (ppm) = 179.7 (C_h), 169.3 (C_g), 132.6 (C_c), 132.1 (C_e), 132.0 (C_b), 131.4 (C_d), 130.1 (C_a), 130.7 (C_f). IR (KBr, cm^{-1}) υ = 3436 (br s, N-H), 1615 (s, C=N), 1560 (m, C=N), 1459 (m, C=C aromatics).

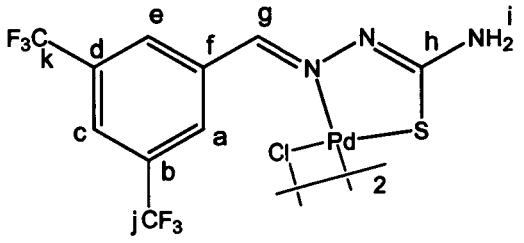
*5.3.3.2. [Pd(3,5-Bis(trifluoromethyl)acetophenone thiosemicarbazone)Cl]$_2$ (**33a**)*



33a

The ligand, 3,5-bis(trifluoromethyl)acetophenone thiosemicarbazone (1. 512 g, 4.593 mmol) was reacted with potassium tetrachloropalladate (1.657 g, 5.076 mmol). The crude product (**33a**) was isolated as a fluffy orange solid (2.057 g, 95 % crude yield). M.p.: decomp. without melting 299-306 °C. ^{1}H NMR (399.95 MHz, DMSO): δ (ppm) = 8.53 (s, 2H, H_a, H_e), 8.19 (s, 1H, H_c), 7.19 (s, 2H, H_j), 1.81 (s, 3H, H_h). ^{19}F NMR (376.9 MHz, DMSO): δ (ppm) = -61.59 (CF_3). IR (KBr, cm^{-1}) υ = 3409 (m, N-H), 3308 (m, N-H), 1614 (s, C=N), 1579 (w, C=N).

*5.3.3.3. [Pd(3,5-Bis(trifluoromethyl)benzaldehyde thiosemicarbazone)Cl]$_2$ (**34a**)*



34a

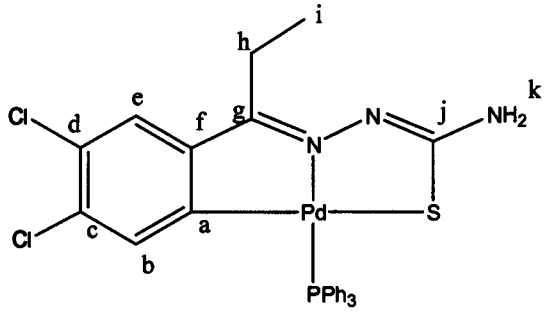
The ligand, 3,5-bis(trifluoromethyl)benzaldehyde thiosemicarbazone (0.448 g, 1.42 mmol) was reacted with potassium tetrachloropalladate (0.507 g, 1.555 mmol). The crude product (**34a**) was isolated as a light orange solid (0.815 g, 63 %, crude yield). M.p.: decomp. without melting 270-276 °C. ^{1}H NMR (399.95 MHz, DMSO): δ (ppm) = 8.71 (s, 2H, H_a, H_e), 8.45 (s, 1H, H_g), 8.12 (s, 1H, H_c), 7.31 (s, 2H, H_i). ^{19}F NMR (376.9 MHz, DMSO): δ (ppm) = -61.69 (CF_3). IR (KBr, cm^{-1}) υ = 3469 (s, N-H), 3279 (s, N-H), 1618 (s, C=N), 1582 (m, C=N).

5.3.4. Preparation of Monomeric Thiosemicarbazone Palladium Complexes

5.3.4.1. Method A: Synthesis from Tetrameric Complexes

The tetrameric complex (1 mol. equiv.) was suspended in dry acetone (15 cm^3). Triphenylphosphine (4 mol. equiv.) was added and the reaction was stirred under argon gas for 4 hours. The product was collected as a bright yellow solid by filtration, washed with acetone (2 x 5 cm^3) and dried under vacuum.

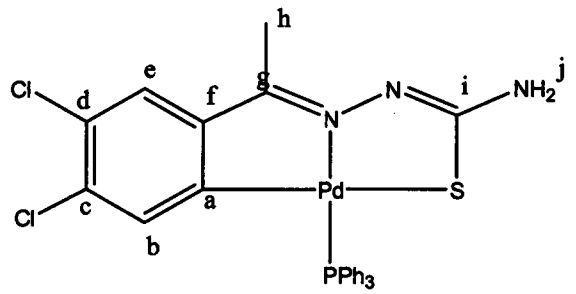
*5.3.4.1.1. [Pd(3,4-Dichloro-propiophenone thiosemicarbazone)(PPh_3)] (**30b**)*



30b

Triphenylphosphine (0.089 g, 0.338 mmol) was reacted with [{3,4-dichloro-propiophenone thiosemicarbazone}Pd]$_4$ (0.106 g, 0.0700 mmol). The product (**30b**) precipitated out of solution as a yellow solid (0.129 g, 72 %). M.p.: 280-283 °C. ^{1}H NMR (300.08 MHz, DMSO): δ (ppm) = 7.40-7.74 (m, 15H, PPh_3), 7.21 (s, 1H, H_b), 6.88 (s, 2H, H_k), 6.34 (s, 1H, H_e), 2.82 (q, J = 7.32, 7.29 Hz, 2H, H_h), 1.11 (t, J = 7.22 Hz, 3H, H_i). ^{13}C NMR (75.46 MHz, DMSO): δ (ppm) = 176.9 (C_j), 168.2 (C_g), 164.1 (C_a), 151.5 (C_f), 136.5 (C_d), 133.9 – 128.6 (C_c and PPh_3), 126.3 (C_e), 125.9 (C_b), 19.5 (C_h), 10.9 (C_i). ^{31}P NMR (121.47 MHz, DMSO): δ (ppm) = 38.55 (PPh_3). IR (KBr, cm^{-1}) υ = 3466 (m, N-H), 3289 (s, N-H), 1618 (s, C=N), 1577 (m, C=N), 1481 (s, C=C aromatics). Elemental Analysis for $C_{28}H_{24}Cl_2N_3PPdS$: found C 52.21, H 3.88, N 5.93, S 4.55 %; calculated C 52.31, H 3.76, N 6.54, S 4.99 %. ESI-MS: *m/z* 644 ([M + H]$^+$, 100%).

*5.3.4.1.2. [Pd(3,4-Dichloro-acetophenone thiosemicarbazone)(PPh_3)] (**31b**)*



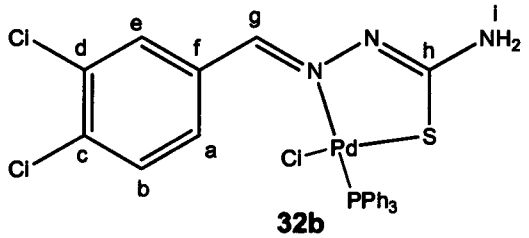
31b

Triphenylphosphine (0.073 g, 0.275 mmol) was reacted with [{3,4-dichloro-aectophenone thiosemicarbazone}Pd]$_4$ (0.100g, 0.069 mmol). The product (**31b**) precipitated out of solution as a yellow solid (0.129 g, 75 %). M.p.: decomposition without melting 290-291 °C. ^{1}H NMR (300.08 MHz, DMSO): δ (ppm) = 7.42-7.64 (m, 15H, PPh_3), 7.20 (s, 1H, H_b), 6.79 (s, 2H, H_j), 6.10 (s,1H, H_e), 2.76 (s, 3H, H_h). ^{13}C NMR (75.46 MHz, DMSO): δ (ppm) = 176.6 (C_i), 163.6 (C_g), 152.7 (C_a), 136.1 (C_f), 133.8 (C_d), 133.7 (C_c), 128.5-131.1 (PPh_3), 126.2 (C_e), 126.1 (C_b), 13.0 (C_h). ^{31}P NMR (121.47 MHz, DMSO): δ (ppm) = 38.75 (PPh_3). IR (KBr, cm^{-1}) υ = 3474 (m, N-H), 3332 (s, N-H), 1601 (s, C=N), 1579 (m, C=N), 1496 (s, C=C aromatics). Elemental Analysis for $C_{27}H_{22}Cl_2N_3PPdS$: found C 51.36, H 3.49, N 6.29, S 4.84 %; calculated C 51.57, H 3.53, N 6.68, S 5.10 %. ESI-MS: *m/z* 630 ([M + H]$^+$, 100%).

5.3.4.2 Method B: Synthesis from Dimeric Complexes

The dimeric complex (1 mol equiv.) was added to a stirred solution of triphenylphosphine (2 mol equiv.) in dry dichloromethane (5 cm^3). The resulting orange reaction mixture was stirred at room temperature for 18 hours, at which time the reaction was a clear orange. Addition of hexane causes the product to precipitate and it is isolated by filtration and washed several times with boiling hexane.

*5.3.4.2.1. [Pd(3,4-Dichloro-benzaldehyde thiosemicarbazone)(PPh_3)Cl] (**32b**)*

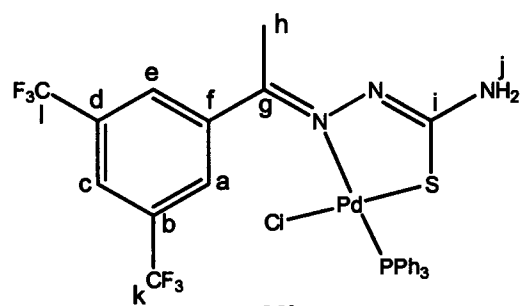


32b

The crude dimeric complex, [(3,4-dichloro-benzaldehyde thiosemicarbazone)$PdCl]_2$ (0.206 g, 0.317 mmol) was reacted with triphenylphosphine (0.169 g, 0.638 mmol) to give the product (**32b**) as a fluffy orange solid (0.101 g, 49 %). M.p.: 249-251 °C. ^{1}H NMR (300.08 MHz, $CDCl_3$): δ (ppm) = 8.47 (m, 2H, H_b, H_e), 7.87 (d, J = 8.56 Hz, 1H, H_g), 7.70-7.80 (m, 6H, PPh_3), 7.40-7.55 (m, 10H, PPh_3, H_a), 4.93 (s, 2H, H_i). ^{13}C NMR (75.46 MHz, $CDCl_3$): δ (ppm) = 175.8 (C_h), 150.9 (C_g), 128.3-134.6 (C_a, C_b, C_c, C_d, C_e, C_f and PPh_3). ^{31}P NMR (121.47 MHz, $CDCl_3$): δ (ppm) = 27.10 (PPh_3). IR (KBr, cm^{-1}) υ = 3468 (m, N-H), 3365 (s, N-H), 1595 (s, C=N), 1581 (m, C=N), 1505 (s, C=C aromatics). Elemental Analysis for $C_{27}H_{23}Cl_3N_3PPdS$: found C 48.76, H 3.18, N 5.67, S 4.65 %; calculated C 48.74, H 3.48, N 6.32, S 4.82 %. ESI-MS: *m/z* 615.95 $[M - Cl]^+$, 100 %.

5.3.4.2.2. [Pd(3,5-Bis(trifluoromethyl)acetophenone thiosemicarbazone)(PPh_3)Cl]

(33b)



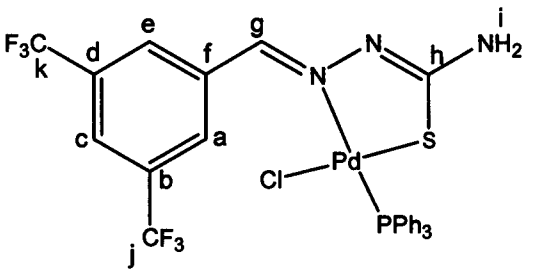
33b

The crude dimeric complex, [(3,5-bis(trifluoromethyl)acetophenone thiosemicarbazone)$PdCl]_2$ (0.204 g, 0.224 mmol), was reacted with triphenylphosphine (0.117g, 0.442 mmol) to give the product (**33b**) as a fluffy orange solid (0.060 g, 37 %). M.p.: 207-209 °C. ^{1}H NMR (300.08 MHz, $CDCl_3$): δ (ppm) = 8.19 (s, 2H, H_a, H_e), 7.93 (s, 1H, H_c), 7.46-7.63 (m, 6H, PPh_3), 7.36-7.45 (m, 9H, PPh_3), 4.77 (s, 2H, H_j) 2.52 (s, 3H, H_h).^{13}C NMR (100.58 MHz, $CDCl_3$): δ (ppm) = 172.1 (C_l), 165.7 (C_g), 142.3 (C_f), 128.0-134.1 (C_a, C_b, C_c, C_d, C_e and PPh_3), 123.1

(C_k, C_l), 21.8 (C_h). ^{19}F NMR (376.9 MHz, $CDCl_3$): δ = -62.93 ($C\underline{F}_3$). ^{31}P NMR (161.90 MHz, $CDCl_3$): δ (ppm) = 29.22 (PPh_3). IR (KBr, cm^{-1}) υ = 3446 (br s, N-H), 1624 (m, C=N), 1603 (m, C=N), 1513 (s, C=C aromatics). Elemental Analysis for $C_{29}H_{23}ClF_6N_3PPdS$: found C 47.86, H 3.28, N 5.37, S 4.20 %; calculated C 47.56, H 3.17, N 5.74, S 4.38 %. ESI-MS: *m/z* 695.98 $[M - Cl]^+$, 100 %.

5.3.4.3.3. [Pd(3,5-Bis(trifluoromethyl)benzaldehyde thiosemicarbazone)(PPh_3)Cl]

(34b)



34b

The crude dimeric complex, [(3,5-bis(trifluoromethyl)benzaldehyde thiosemicarbazone)$PdCl]_2$ (0.201 g, 0.252 mmol), was reacted with triphenylphosphine (0.136 g, 0.519 mmol) to give the product (**34b**) as an orange solid (0.090 g, 46 %) . M.p.: 135-136 °C. ^1H NMR (399.95 MHz, $CDCl_3$): δ (ppm) = 8.69 (s, 1H, H_g), 8.66 (s, 2H, H_a, H_e), 7.89 (s, 1H, H_c), 7.74-7.80 (m, 6H, PPh_3), 7.45-7.54 (m, 9H, PPh_3), 5.01 (s, 2H, H_i). ^{13}C NMR (100.58 MHz, $CDCl_3$): δ (ppm) = 177.0 (C_h), 149.6 (C_g), 128.4-134.6 (C_a, C_b, C_c, C_d, C_e, C_f and PPh_3), 123.7 (C_j, C_k).^{19}F NMR (376.9 MHz, $CDCl_3$): δ(ppm) = -63.38 ($C\underline{F}_3$). ^{31}P NMR (161.90 MHz, $CDCl_3$): δ (ppm) = 27.74 (PPh_3). IR (KBr, cm^{-1}) υ = 3461 (s, N-H), 3341 (s, N-H), 1607 (s, C=N), 1577 (w, C=N), 1491 (s, C=C aromatics). Elemental Analysis for $C_{28}H_{21}ClF_6N_3PPdS$: found C 48.65, H 3.25, N 4.78, S 3.83 %; calculated C 46.81, H 2.95, N 5.85, S 4.46 ESI-MS: *m/z* 681.99 $[M - Cl]^+$, 100 %.

5.4. X-ray Crystallography

X-ray single crystal intensity data were collected on a Nonius Kappa-CCD diffractometer using graphite monochromated MoKα radiation. Temperature was controlled by an Oxford Cryostream cooling system (Oxford Cryostat). The strategy

for the data collections was evaluated using the Bruker Nonius "Collect" program. Data were scaled and reduced using DENZO-SMN software.[11] For complexes **30b** and **32b**, empirical absorption corrections using the program SADABS (Sheldrick, 1996) were applied.[12]

For all complexes, the structures were solved by direct methods and refined employing full-matrix least-squares with the program SHELXL-97[13] refining on F^2. Packing diagrams were produced using the program PovRay and graphic interface X-seed.[14] and all non-H atoms were refined anisotropically. All hydrogen atoms, except the amino hydrogens H1A and H1B for complexes **26a**, **27a** and **28a** and H1N and H2N for **30b**, were included in idealised positions in a riding model with U_{iso} set at 1.2 or 1.5 times those of the parent atoms. The amino hydrogens were located by difference Fourier methods and refined independently with simple bond length constraint.

5.4. Biological Experiments

5.4.1 Anticancer Experiments

5.4.1.1 Cell lines

The WHCO1 cell line was derived from biopsies of primary oesophageal squamous cell carcinomas [15] and kindly provided by Professor Rob Veale (University of Witwatersrand, South Africa). KYSE - oesophageal squamous cell carcinoma cell lines previously established by Shimada and co-workers [16] were purchased from the German Resource Centre for Biological Material (http://www.dsmz.de). The cervical cancer cell lines HeLa and CaSki were obtained from the American Type Culture Collection (Rockville, MD, USA). All cultures were maintained in Dulbecco's Modified Eagle Medium containing 10% Foetal Calf Serum and 100U/ml streptomycin and 100µg/ml penicillin. Cells were maintained in a humidified 37°C, 5% CO_2 incubator.

5.4.1.2. IC_{50} Determination

IC_{50} determinations were carried out using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Briefly, 3000 cells were seeded per well in 96-

well plates. Cells were incubated at 37°C and 5% CO_2 (24 h), after which aqueous DMSO solutions of each compound (10µL, with a constant final concentration of DMSO = 0.2%) were plated at various concentrations. After 48 h incubation, observations were made, and MTT (10µL) solution added to each well. After a further 4 h incubation, solubilization solution (100µL) was added to each well, and plates were incubated overnight. Plates were read at 595nm on a BioTek microplate reader. WHCO1 (oesophageal cancer cells of South African origin) cells were used.

5.4.1.3. Western Blot Analysis

Cells (0.5 x 10^6) were plated in 60mm dishes and incubated for 24 h, after which cells were exposed to concentrations of thiosemicarbazone **27** ranging from 0 to 20µM for 48 h. Cells were harvested in 60µl of lysis buffer and following sonication, protein was quantitated using the Pierce BCA kit. Equal amounts of protein were loaded on a 10% polyacrylamide gel and subjected to electrophoresis at a constant current of 15 mA. After transfer at 100V for 1 h, membranes were blocked with 5% fat-free milk in tris-buffered saline with 0.1% Tween 20 (TBS/T) for 1 h at room temperature. After overnight incubation at 4°C with the primary antibody PARP (1:1000, 5% milk) and β-tubulin (1:1000, TBS/Tween) the membrane was incubated with horseradish peroxidase-conjugated antirabbit secondary antibody for 1 h at room temperature. Detection using Extended West Dura Super Signal (Pierce) was carried out and bands were visualized by autoradiography.

5.4.2. Antimalarial Testing

5.4.2.1. Chloroquine Sensitive (CQS) Strain of Plasmodium falciparum (D10)

The test compounds were tested in triplicate on one occasion. Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen.[17] Quantitative assessment of antiplasmodial activity in vitro was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler.[18] The samples were prepared to a 2 mg/ml stock solution in 10% DMSO and sonicated to enhance solubility. Samples were tested as a suspension if not completely dissolved. Stock solutions were stored at -

20°C. Further dilutions were prepared on the day of the experiment. Chloroquine (CQ) was used as the reference drug in all experiments. The percentage parasite survival was determined for all test samples at a single concentration (10 µg/ml). Thereafter, a full dose-response was performed for all compounds showing a percentage survival less than 50 % to determine the concentration inhibiting 50% of parasite growth (IC_{50}–value). Test samples were tested at a starting concentration of 100 µg/ml, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 0.2 µg/ml. Complex **30b** was tested at a starting concentration of 10 µg/ml. The same dilution technique was used for all samples. CQ was tested at a starting concentration of 100 ng/ml. The highest concentration of solvent to which the parasites were exposed to had no measurable effect on the parasite viability (data not shown). The IC_{50}-values were obtained using a non-linear dose-response curve fitting analysis via Graph Pad Prism v.4.[19]

5.4.2.2. Chloroquine Resistant (CQR) Strain of Plasmodium falciparum (W2) [19]

Ring stage, W2-strain *P. falciparum* parasites (1% parasitaemia, 2% haematocrit) were cultured in 0.5 mL of medium in 48-well culture dishes. Inhibitors from 10 mM stocks in DMSO were added to cultured parasites to give a final concentration of 20 µM. From 48-well plates, 125 µM of culture was transferred to two 96 well plates (duplicates). Serial dilutions (1:5) of inhibitors were made to final concentrations of 10 µM, 2 µM, 0.4 µM, 80 nM, 16 nM and 3.2 nM. Cultures were maintained at 37°C for 2 days after which the parasites were washed and fixed with 1% formaldehyde in PBS. After two days, parasitaemia was measured by flow cytometry using the DNA stain YOYO-1 as a marker for cell survival. IC_{50}-values for growth inhibition were determined with GraphPad Prism software from plots of percentage parasitemia of untreated control cultures against inhibitor concentration.

5.5. General Procedure for Catalytic Experiments

The aryl bromide (1 mmol), phenylboronic acid (1.5 mmol), sodium carbonate (2 mmol) and water (1 ml) were added to 2ml of DMF in a round-bottomed flask fitted with a reflux condensor. A 1 mM stock solution of the palladium complex in DMF (1 ml) was then added and the resulting reaction mixture was stirred at 100°C for 24

hours. Upon cooling to room temperature, distilled water was added and the products extracted with DCM. The organic phase was washed with brine and then dried over sodium sulphate, filtered and passed through celite. The volume of the organic phase was then concentrated to approximately 5 ml and the products analysed using gas chromatography. The conversions of the aryl bromide to product were calculated based on decane (0.1 ml) as internal standard.

5.6. References

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